

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/435, 14/705, C09K 11/06, G01N 33/53, 33/566	A1	(11) International Publication Number: WO 99/18124 (43) International Publication Date: 15 April 1999 (15.04.99)
(21) International Application Number: PCT/US98/21049 (22) International Filing Date: 6 October 1998 (06.10.98) (30) Priority Data: 60/061,385 7 October 1997 (07.10.97) US (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CUMMINGS, Richard, T. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). HERMES, Jeffrey, D. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). MOLLER, David, E. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). ZHOU, Gaochao [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: ASSAYS FOR NUCLEAR RECEPTOR LIGANDS USING FRET (57) Abstract Provided is a method of identifying agonists and antagonists of nuclear receptors that comprises measuring agonist-dependent fluorescence resonance energy transfer (FRET) between a fluorescently labeled nuclear receptor or ligand binding domain and fluorescently labeled CREB-binding protein (CBP), p300, other nuclear co-activator, or binding portion thereof. The method is simple, rapid, and inexpensive. Nuclear receptors and nuclear receptor co-activators labeled with fluorescent reagents for use in the above-described method are also provided.		

B2

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

TITLE OF THE INVENTION

ASSAYS FOR NUCLEAR RECEPTOR LIGANDS USING FRET

5

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/061,385, filed 10/7/97, the contents of which are incorporated herein by reference in their entirety.

10

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

15

Not applicable.

FIELD OF THE INVENTION

20

This invention relates to methods of identifying novel agonists and antagonists of nuclear receptors utilizing the agonist-dependent interaction of such receptors with CREB-binding protein (CBP) or other nuclear receptor co-activators in which this interaction is detected by fluorescence resonance energy transfer.

BACKGROUND OF THE INVENTION

25

30

35

Nuclear receptors are a superfamily of ligand-activated transcription factors that bind as homodimers or heterodimers to their cognate DNA elements in gene promoters. The superfamily, with more than 150 members, can be divided into subfamilies (*e.g.* the steroid, retinoid, thyroid hormone, and peroxisome proliferator-activated [PPAR] subfamilies). Each subfamily may consist of several members which are encoded by individual genes (*e.g.* PPAR α , PPAR γ , and PPAR δ). In addition, alternative mRNA splicing can result in more than one isoform of these genes as in the case of specific PPARs (*e.g.* PPAR γ 1 and PPAR γ 2). The nuclear receptor superfamily is involved in a wide variety of physiological functions in mammalian cells: *e.g.*, differentiation, proliferation, and metabolic homeostasis. Dysfunction

or altered expression of specific nuclear receptors has been found to be involved in disease pathogenesis.

The PPAR subfamily of nuclear receptors consists of three members: PPAR α , PPAR γ , and PPAR δ . PPAR α is highly expressed in liver and kidney. Activation of PPAR α by peroxisome proliferators (including hypolipidemic reagents such as fibrates) or medium and long-chain fatty acids is responsible for the induction of acyl-CoA oxidase and hydratase-dehydrogenase (enzymes required for peroxisomal β -oxidation), as well as cytochrome P450 4A6 (an enzyme required for fatty acid ω -hydroxylase). Thus, PPAR α has an important role in the regulation of lipid metabolism and is part of the mechanism through which hypolipidemic compounds such as fibrates exert their effects. PPAR γ is predominantly expressed in adipose tissue. Recently, a prostaglandin J2 metabolite, 15-Deoxy-D12,14-prostaglandin J2, has been identified as a potential physiological ligand of PPAR γ . Both 15-Deoxy-D12,14-prostaglandin J2 treatment of preadipocytes or retroviral expression of PPAR γ 2 in fibroblasts induced adipocyte differentiation, demonstrating the role of PPAR γ in adipocyte differentiation and lipid storage. The demonstration that anti-diabetic and lipid-lowering insulin sensitizing compounds known as thiazolidinediones are high affinity ligands for PPAR γ suggests a broad therapeutic role for PPAR γ ligands in the treatment of diabetes and disorders associated with insulin resistance (*e.g.* obesity and cardiovascular disease).

Nuclear receptor proteins contain a central DNA binding domain (DBD) and a COOH-terminal ligand binding domain (LBD). The DBD is composed of two highly conserved zinc fingers that target the receptor to specific promoter/enhancer DNA sequences known as hormone response elements (HREs). The LBD is about 200-300 amino acids in length and is less well conserved than the DBD. There are at least three functions for the LBD: dimerization, ligand binding, and transactivation. The transactivation function can be viewed as a molecular switch between a transcriptionally inactive and a transcriptionally active state of the receptor. Binding of a ligand which is an agonist flips the switch from the inactive state to the active state. The COOH-terminal portion of the LBD contains an activation function domain (AF2) that is required for the switch.

The ligand-induced nuclear receptor molecular switch is mediated through interactions with members of a family of nuclear receptor co-activators (*e.g.*, CBP/p300, SRC-1/NcoA-1, TIF2/GRIP-1/NcoA-2, and p/CIP). Upon binding of agonist to its cognate receptor LBD, a conformational change in the receptor protein creates a co-activator binding surface and results in recruitment of co-activator(s) to the receptor and subsequent transcriptional activation. The binding of antagonist ligands to nuclear receptors will not induce the required conformational change and prevents recruitment of co-activator and subsequent induction of transcription. The co-activators CREB-binding protein (CBP) and p300 are two closely related proteins that were originally discovered by virtue of their ability to interact with the transcription factor CREB. These two proteins share extensive amino acid sequence homology. CBP can form a bridge between nuclear receptors and the basic transcriptional machinery (Kamei et al., 1996, Cell 85:403-414; Chakravarti et al., 1996, Nature 383:99-103; Hanstein et al., 1996, Proc. Natl. Acad. Sci. USA 93:11540-11545; Heery et al., 1997, Nature 387:733-736). CBP also contains intrinsic histone acetyltransferase activity which could result in local chromatin rearrangement and further activation of transcription. Ligand- and AF2-dependent interaction between certain nuclear receptors and CBP has been demonstrated in *in vitro* pull down assays and far-western assays. This interaction is both necessary and sufficient for the transcriptional activation that is mediated by these nuclear receptors. Thus, an AF2 mutant of the estrogen receptor (ER) which abolishes the transcriptional function of the receptor is incapable of interacting with CBP.

The N-termini of CBP and p300 have been shown to interact with the ligand-binding domains of some nuclear receptors (Kamei et al., 1996, Cell 85:403-414, hereinafter "Kamei"). Kamei was able to demonstrate direct interaction of CBP and p300 with nuclear receptors by several different methods:

(1) Kamei produced GST fusion proteins of the first 100 amino acids of the N-terminus of CBP. These fusion proteins were run out on a polyacrylamide gel, transferred to a membrane, and the membrane was exposed to ³²P-labeled ligand-binding domains of

nuclear receptors. In the presence of ligand, a specific binding interaction between the CBP and nuclear receptor fragments was detected in that the ^{32}P -labeled ligand-binding domains were observed to bind to the bands on the membrane containing the GST-CBP fusion proteins.

(2) Kamei also utilized the yeast two-hybrid system. The ligand-binding domain of the nuclear receptor fused to the DNA-binding domain of the LexA protein was used as bait. The amino terminal domain of CBP fused to the gal4 transactivation domain was used as prey. In the presence of ligand, a specific binding interaction (occurring *in vivo*, i.e., within the yeast) was observed between the CBP and nuclear receptor fragments.

(3) Kamei observed ligand-induced binding between CBP and nuclear receptors via a gel-shift assay. This assay is based on the observation that, in the presence of ligand, nuclear receptors will bind to oligonucleotides containing their target recognition sequence. Such binding results in the formation of a nuclear receptor-ligand-oligonucleotide complex having a higher molecular weight than the oligonucleotide alone. This difference in molecular weight is detected via a shift in position of the ^{32}P -labeled oligonucleotide when it is run out on a polyacrylamide gel. Kamei found that a fragment of CBP (the N-terminal 100 amino acids) was capable of binding to the nuclear receptor-ligand-oligonucleotide complex and shifting the complex's position on the gel to an even higher molecular weight.

(4) Kamei was able to co-immunoprecipitate CBP using antibodies to nuclear receptors in extracts from a variety of cells in the presence of ligand.

(5) By the use of transcriptional activation assays, Kamei was able to demonstrate that nuclear receptors and CBP interact in a functional manner. Such transcriptional activation assays can indicate that two proteins are involved in a pathway that results in transcriptional activation but these assays do not prove that the interaction between the proteins is one of direct binding.

By the above-described methods, Kamei was able to demonstrate specific binding interactions between CBP and the retinoic acid receptor (RAR), glucocorticoid receptor (GR), thyroid hormone

receptor (T3R), and retinoid X receptor (RXR). Kamei also demonstrated specific binding between the N-terminus of p300 and RAR. However, Kamei did not demonstrate specific binding between CBP, p300, or any other nuclear receptor co-activators and PPARs.

5 What is striking about the methods used by Kamei is their extremely laborious and time consuming nature. Such methods involve, among other things, the construction of fusion proteins, the preparation of ³²P-labeled proteins, the construction of specialized expression vectors for the yeast two-hybrid assay and the transcriptional
10 activation assays, the running of many gels, and the raising of antibodies. Most of these assays take days to carry out and preparing the reagents needed to carry them out may take weeks. Because of the complicated reagents that are involved in these assays and the time needed to prepare and run the assays, these assays tend to be costly.
15 Investigators other than Kamei who have studied the interaction between nuclear receptors and CBP have also been forced to rely on such cumbersome methods (see, *e.g.*, Chakravarti et al., 1996, Nature 383:99-103; Hanstein et al., 1996, Proc. Natl. Acad. Sci. USA 93:11540-11545; Heery et al., 1997, Nature 387:733-736).
20 Kamei did not use the above-described methods to identify novel agonists or antagonists of nuclear receptors. The focus of Kamei was not on agonists or antagonists, but rather on the interaction between nuclear receptors and CBP. Although modifying the methods of Kamei to identify agonists or antagonists might be possible, such
25 methods would suffer from serious disadvantages. This is because, as discussed above, all of the assays employed by Kamei to study the interaction of CBP and p300 with nuclear receptors are very laborious, slow, and costly. Given the therapeutic importance of steroid hormones such as estrogen, cortisol, progesterone, and other nuclear receptor
30 agonists such as thyroid hormone and antidiabetic thiazolidinedione compounds, the need for improved high-throughput screening assays to identify potential pharmaceutical compounds affecting nuclear receptors is clear. Historically, therapeutically useful nuclear receptor ligand compounds were identified by screening animal models, an
35 approach which is even more labor intensive and time consuming than the methods used by Kamei. Also, approaches such as those used by

Kamei are ill-suited for the identification of antagonists of nuclear receptors. It is now widely appreciated that antagonists of nuclear receptors can be valuable therapeutic agents. Examples of such therapeutically useful antagonists are tamoxifene, raloxifene, and RU-486.

What is needed is a high throughput, time and labor-saving, non-radioactive, inexpensive, and very reliable assay for the identification and characterization of both agonists and antagonists of nuclear receptors. Such an assay is provided by the present invention.

SUMMARY OF THE INVENTION

The present invention provides novel methods of identifying agonists and antagonists of nuclear receptors. The methods take advantage of the agonist-dependent binding of nuclear receptors and CBP, p300, or other nuclear receptor co-activators. In the absence of agonist, binding between the nuclear receptor and CBP, p300, or other nuclear receptor co-activators does not occur. If agonist is present, however, such binding occurs and can be detected by fluorescence resonance energy transfer (FRET) between a fluorescently-labeled nuclear receptor and fluorescently-labeled CBP, p300, or other nuclear receptor co-activator. Antagonists can be identified by virtue of their ability to prevent or disrupt the agonist-induced interaction of nuclear receptors and CBP, p300, or other nuclear receptor co-activators. In contrast to prior art methods of identifying agonists and antagonists of nuclear receptors, the methods of the present invention, are simple, rapid, and less costly.

The present invention provides a nuclear receptor or ligand binding domain thereof labeled with a fluorescent reagent for use in the above-described methods of identifying agonists and antagonists of nuclear receptors. The present invention also provides CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a fluorescent reagent.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a method of fluorescently labelling a protein or polypeptide with Europium cryptate (Eu³+K).

5 Figure 2 illustrates the format for experiments 1 and 2 of Table 1.

 Figure 3 illustrates the format for experiment 3 of Table 1.

10 Figure 4 illustrates the format for experiment 4 of Table 1.

 Figure 5 shows the results of studies using the methods of the present invention with four known PPAR γ agonists. --o-- = AD5075; --□-- = Pioglitazone; --X-- = Troglitazone; --◇-- = BRL49653.

15 Figure 6 shows a measurement of the binding constant for the interaction between hCBP and PPAR γ 1LBD.

 Figure 7A shows the amino acid sequence of human CBP (SEQ.ID.NO.:1).

20 Figure 7B shows the nucleotide sequence of a cDNA encoding human CBP (SEQ.ID.NO.:2). The open reading frame is at positions 76-1290.

 Figure 8A shows the amino acid sequence of human PPAR α (SEQ.ID.NO.:3).

25 Figure 8B shows the nucleotide sequence of a cDNA encoding human PPAR α (SEQ.ID.NO.:4). The open reading frame is at positions 217-1623.

 Figure 9A shows the amino acid sequence of human PPAR γ 1 (SEQ.ID.NO.:5).

30 Figure 9B shows the nucleotide sequence of a cDNA encoding human PPAR γ 1 (SEQ.ID.NO.:6). The open reading frame is at positions 173-1609.

 Figure 10A shows the amino acid sequence of human PPAR δ (SEQ.ID.NO.:7).

35 Figure 10B-C shows the nucleotide sequence of a cDNA encoding human PPAR δ (SEQ.ID.NO.:8). The open reading frame is at positions 338-1663.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

- an "agonist" is a substance that binds to nuclear receptors in such a way that a specific binding interaction between the nuclear receptor and CBP or other nuclear receptor co-activator can occur.

- an "antagonist" is a substance that is capable of preventing or disrupting the agonist-induced specific binding interaction between a nuclear receptor and CBP, p300, or another nuclear receptor co-activator.

- a "ligand" of a nuclear receptor is an agonist or an antagonist of the nuclear receptor.

- a "specific binding interaction," "specific binding," and the like, refers to binding between a nuclear receptor and CBP, p300, or other nuclear receptor co-activator which results in the occurrence of fluorescence resonance energy transfer between a fluorescent reagent bound to the nuclear receptor and a fluorescent reagent bound to CBP, p300, or other nuclear receptor co-activator.

With respect to CBP, p300, or other nuclear receptor co-activators, a "binding portion" is that portion of CBP, p300, or other nuclear receptor co-activators that is sufficient for specific binding interactions with nuclear receptors.

With respect to nuclear receptors, a "ligand binding domain" is that portion of a nuclear receptor that is sufficient to bind an agonist or antagonist of the nuclear receptor.

The present invention provides a high throughput, time and labor-saving, non-radioactive, inexpensive, and very reliable assay for the identification and characterization of both agonists and antagonists of nuclear receptors. In a general embodiment, the present invention provides methods of identifying agonists and antagonists for any nuclear receptor for which CBP, p300, or another nuclear receptor binding protein is a co-activator. Such agonists and antagonists are identified by virtue of their ability to induce or prevent binding between the ligand binding domain of a nuclear receptor and CBP, p300, or other nuclear receptor co-activator. The interaction between the nuclear receptor and CBP, p300, or other nuclear receptor co-activator is monitored by observing the occurrence of fluorescence resonance energy

transfer (FRET) between two fluorescent reagents. One fluorescent reagent is bound to the nuclear receptor; the other fluorescent reagent is bound to CBP, p300, or other nuclear receptor co-activator. The binding of fluorescent reagent to nuclear receptor, CBP, p300, or other nuclear receptor co-activator can be by a covalent linkage or a non-covalent linkage.

The present invention makes use of fluorescence resonance energy transfer (FRET). FRET is a process in which energy is transferred from an excited donor fluorescent reagent to an acceptor fluorescent reagent by means of intermolecular long-range dipole-dipole coupling. FRET typically occurs over distances of about 10 \AA to 100 \AA and requires that the emission spectrum of the donor reagent and the absorbance spectrum of the acceptor reagent overlap adequately and that the quantum yield of the donor and the absorption coefficient of the acceptor be sufficiently high. In addition, the transition dipoles of the donor and acceptor fluorescent reagents must be properly oriented relative to one another. For a review of FRET and its applications to biological systems, see Clegg, 1995, Current Opinions in Biotechnology 6:103-110.

The present invention makes use of a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent. The second fluorescent reagent comprises a fluorophore capable of undergoing energy transfer by either (a) donating excited state energy to the first fluorescent reagent, or (b) accepting excited state energy from the first fluorescent reagent. In other words, according to the present invention, either the first or the second fluorescent reagents can be the donor or the acceptor during FRET.

The first and second fluorescent reagents are spectroscopically complementary to each other. This means that their spectral characteristics are such that excited state energy transfer can occur between them. FRET is highly sensitive to the distance between the first and second fluorescent reagents. For example, FRET varies inversely with the sixth power of the distance between the first and second fluorescent reagents. In the absence of agonist, the first

fluorescent reagent, bound to the nuclear receptor or ligand binding domain thereof, will not be near the second fluorescent reagent, bound to CBP, p300, or other nuclear receptor co-activator, or binding portion thereof. Thus, no FRET, or very little FRET, will be observed. In the presence of agonist, however, interaction between the nuclear receptor and CBP, p300, or other nuclear receptor co-activator will occur, thus bringing close together the first and the second fluorescent reagents, allowing FRET to occur and be observed.

Accordingly, the present invention provides a method of identifying an agonist of a nuclear receptor that comprises providing:

- (a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
- (b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent; and
- (c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, will occur; and

- (d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

In particular embodiments, the nuclear receptor is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, and "orphan nuclear receptors" such as LXR, FXR, etc.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof is a ligand binding domain of a nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof comprises an AF-2 site of a nuclear receptor.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length PPAR. In another embodiment, the nuclear receptor or ligand binding domain thereof is the ligand binding domain of a PPAR. In a further embodiment, the PPAR is
5 selected from the group consisting of PPAR α , PPAR γ 1, PPAR γ 2, and PPAR δ . In a further embodiment, the ligand binding domain of the PPAR contains amino acid residues 176-478 of human PPAR γ 1.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 143-462 of human RAR α .

10 In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 122-410 of rat T3R α 1. In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 227-463 of mouse RXR γ . In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino
15 acids 251-595 of human ER.

In a particular embodiment, the above-described methods utilize full-length CBP, either mouse or human. In other embodiments, the methods utilize amino acid residues 1-113 of human CBP. In another embodiment, the methods utilize amino acid residues 1-453 of
20 human CBP.

The conditions under which the methods described above are carried out are conditions that are typically used in the art for the study of protein-protein interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as
25 PBS; a temperature of about 4°C to about 55°C. The presence of commonly used non-ionic detergents, *e.g.*, NP-40®, sarcosyl, Triton X-100®, is optional. When europium cryptates are used as fluorescent reagents, reactions should contain KF at a concentration of at least 200 mM.

30 Heery et al., 1997, Nature 387:733-736 showed that interactions between nuclear receptors and a variety of nuclear receptor co-activators are mediated by a short amino acid sequence in the nuclear receptor co-activators having the amino acid sequence LXXLL, where L is leucine and X represents any amino acid. Accordingly, the present
35 invention can be practiced with a binding portion of a nuclear receptor co-activator, provided that the binding portion contains the amino acid

sequence LXXLL. Therefore, the present invention includes a method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

5 (b) a binding portion of a nuclear receptor co-activator, where the binding portion contains the amino acid sequence LXXLL, and where the binding portion is labeled with a second fluorescent reagent; and

10 (c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the binding portion of the nuclear receptor co-activator will take place; and

15 (d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

20 In a particular embodiment, the nuclear receptor co-activator is selected from the group consisting of: human RIP-140, human SRC-1, mouse TIF-2, human or mouse CBP, human or mouse p300, mouse TIF-1, and human TRIP proteins.

25 In a particular embodiment, the nuclear receptor co-activator is human RIP-140 and the binding portion includes a contiguous stretch of amino acids of human RIP-140 selected from the group consisting of: positions 20-29, 132-139, 184-192, 266-273, 379-387, 496-506, 712-719, 818-825, 935-944, and 935-942.

30 In another embodiment, the nuclear receptor co-activator is human SRC-1 and the binding portion includes a contiguous stretch of amino acids of human SRC-1 selected from the group consisting of: positions 45-53, 632-640, 689-696, 748-755, and 1434-1441.

35 In another embodiment, the nuclear receptor co-activator is mouse TIF-2 and the binding portion includes a contiguous stretch of amino acids of mouse TIF-2 selected from the group consisting of: positions 640-650, 689-699, and 744-754.

In another embodiment, the nuclear receptor co-activator is human or mouse CBP and the binding portion includes a contiguous stretch of amino acids of human or mouse CBP selected from the group consisting of: positions 68-78 and 356-366.

5 In another embodiment, the nuclear receptor co-activator is human or mouse p300 and the binding portion includes a contiguous stretch of amino acids of human or mouse p300 selected from the group consisting of: positions 80-90 and 341-351.

10 In another embodiment, the nuclear receptor co-activator is mouse TIF-1 and the binding portion includes a contiguous stretch of amino acids of mouse TIF-1 containing positions 722-732.

In another embodiment, the nuclear receptor co-activator is human TRIP2 and the binding portion includes a contiguous stretch of amino acids of human TRIP2 containing positions 23-33.

15 In another embodiment, the nuclear receptor co-activator is human TRIP3 and the binding portion includes a contiguous stretch of amino acids of human TRIP3 containing positions 97-107.

In another embodiment, the nuclear receptor co-activator is human TRIP4 and the binding portion includes a contiguous stretch of amino acids of human TRIP4 containing positions 36-46.

20 In another embodiment, the nuclear receptor co-activator is human TRIP5 and the binding portion includes a contiguous stretch of amino acids of human TRIP5 containing positions 26-36.

In another embodiment, the nuclear receptor co-activator is human TRIP8 and the binding portion includes a contiguous stretch of amino acids of human TRIP8 containing positions 36-46.

25 In another embodiment, the nuclear receptor co-activator is human TRIP9 and the binding portion includes a contiguous stretch of amino acids of human TRIP9 selected from the group consisting of: positions 73-83, 256-266 and 288-298.

30 For amino acid sequences of nuclear receptor co-activators, see Yao et al., 1996, Proc. Natl. Acad. Sci. USA 93:10626-10631 (SRC-1); Oşate et al., 1995, Science 270:1354-1357 (SRC-1); Cavailles et al., 1995, EMBO J. 14:3741-3751 (RIP-140); Voegel et al., 1996, EMBO J. 15:101-108 (TIF-2); Kwok et al., 1994, Nature 370:223-226 (CBP); Arias et al., 1994, Nature 370:226-229 (CBP); Eckner et al., 1994, Genes Dev. 8:869-884

(p300); Le Douarin et al., 1995, EMBO J. 14:2020-2033 (TIF-1); Lee et al., 1995, Nature 374:91-94 (TRIP proteins).

The particular embodiments of the present invention described above are all particular embodiments of a more general method that is also part of the present invention. That general method is a method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) a polypeptide containing the amino acid sequence LXXLL where the polypeptide is labeled with a second fluorescent reagent; and

(c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the polypeptide will take place; and

(d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

In a particular embodiment, the amino acid sequence LXXLL is present in an α helical portion of the polypeptide. In another embodiment, the amino acid sequence LXXLL is present in an α helical portion of the polypeptide and the leucines form a hydrophobic face.

The present invention provides methods for identifying antagonists of a nuclear receptor. Such methods are based on the ability of the antagonist to prevent the occurrence of agonist-induced binding between a nuclear receptor and CBP, p300, or other nuclear receptor co-activator, or to disrupt such binding after it has occurred. Thus, the present invention provides a method for identifying antagonists of nuclear receptors that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent;

(c) an agonist of the nuclear receptor; and

(d) a substance suspected of being an antagonist of the nuclear receptor;

5 under conditions such that, in the absence of the substance, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof will occur; and

(e) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents when the
10 substance is present and measuring FRET between the first and second fluorescent reagents when the substance is absent;

where the a decrease in FRET when the substance is present indicates that the substance is an antagonist of the nuclear receptor.

15 In particular embodiments, the nuclear receptor is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, and "orphan nuclear receptors" such as LXR, FXR, etc.

20 In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof is a ligand binding domain of a nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof is an AF-2 site of
25 a nuclear receptor.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length PPAR. In another embodiment, the nuclear receptor or ligand binding domain thereof is the ligand binding domain of a PPAR. In a further embodiment, the PPAR is
30 selected from the group consisting of PPAR α , PPAR γ , and PPAR δ . In a further embodiment, the ligand binding domain of the PPAR contains amino acid residues 176-478 of human PPAR γ 1.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 143-462 of human RAR α .
35 In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 122-410 of rat T₃R α 1. In another

embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 227-463 of mouse RXR γ . In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 251-595 of human ER.

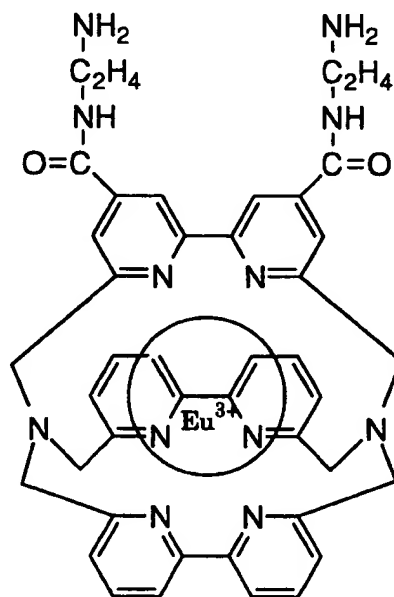
5 In a particular embodiment, the above-described methods utilize full-length CBP, either mouse or human. In other embodiments, the methods utilize amino acid residues 1-113 of human CBP. In another embodiment, the methods utilize amino acid residues 1-453 of human CBP.

10 The conditions under which the methods described above are carried out are conditions that are typically used in the art for the study of protein-protein interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS; a temperature of about 4°C to about 55°C. The presence of
15 commonly used non-ionic detergents, *e.g.*, NP-40®, sarcosyl, Triton X-100®, is optional. When europium cryptates are used as fluorescent reagents, reactions should contain KF at a concentration of at least 200 mM.

In principle, one could measure FRET by monitoring either
20 (a) a decrease in the emission of the donor fluorescent reagent following stimulation at the donor's absorption wavelength and/or (b) an increase in the emission of the acceptor reagent following stimulation at the donor's absorption wavelength. In practice, FRET is most effectively measured by emission ratioing. Emission ratioing monitors the change
25 in the ratio of emission by the acceptor over emission by the donor. An increase in this ratio signifies that energy is being transferred from donor to acceptor and thus that FRET is occurring. Emission ratioing can be measured by employing a laser-scanning confocal microscope. Emission ratioing is preferably done by splitting the emitted light from a
30 sample with a dichroic mirror and measuring two wavelength bands (corresponding to the donor and the acceptor emission wavelengths) simultaneously with two detectors. Alternatively, the emitted light can be sampled consecutively at each wavelength (by using appropriate filters) with a single detector. In any case, these and other methods of
35 measuring FRET are well known in the art.

Although a variety of donor and acceptor fluorescent reagents can be used in the practice of the present invention, preferred embodiments of the present invention make use of cryptates of fluorescent reagents as donor reagents. Inclusion of a substrate into the intramolecular cavity of a macropolycyclic ligand results in the formation of a cryptate. The macropolycyclic ligand shields the substrate from interaction with solvent and other solute molecules. If the substrate is a fluorescent reagent, formation of a cryptate may result in markedly different spectroscopic characteristics for the reagent as compared to the spectroscopic characteristics of the free reagent.

The present invention includes the use of europium (Eu^{III}) or terbium (Tb^{III}) cryptates as donor fluorescent reagents. Such Eu^{III} or Tb^{III} cryptates, as well as methods for their formation, are well known in the art. For example, see Alpha et al., 1987, Angew. Chem. Int. Ed. Engl. 26:266-267; Mathis, 1995, Clin. Chem. 41:1391-1397. A europium cryptate is formed by the inclusion of a europium ion into the intramolecular cavity of a macropolycyclic ligand which contains bipyridine groups as light absorbers. When europium cryptates are present in solution together with fluoride ions, a total shielding of the europium cryptate fluorescence occurs. The molecular structure of a europium cryptate is shown below.



Europium cryptates can be conjugated to proteins by the use of well-known heterobifunctional reagents (see, *e.g.*, International Patent Application WO 89/05813; Prat et al., 1991, Anal. Biochem. 195:283-289; Lopez et al., 1993, Clin. Chem. 39:196-201).

5 The present invention includes the use of XL665 as the acceptor fluorescent reagent. XL665 is a crosslinked derivative of allophycocyanin (APC). APC is a porphyrin containing protein which is derived from the light harvesting system of algae (Kronick, 1986, M. Immunol. Meth. 92:1-13). XL665 has an absorption maximum at ≈ 620
10 nm and an emission maximum at 665 nm. In some embodiments of the invention, XL665 is labeled with streptavidin in order to effect the binding of the streptavidin-labeled XL665 to a biotin-labeled substance, *e.g.*, CBP or the ligand binding domain of a nuclear receptor. Streptavidin labeling of XL655 and biotin labeling of CBP, or the ligand
15 binding domain of a nuclear receptor, can be performed by well known methods.

 In a preferred embodiment of the invention, XL665 as the acceptor fluorescent reagent is combined with Europium cryptate (Eu3+K) as the donor fluorescent reagent. Europium cryptate (Eu3+K)
20 has a large Stokes shift, absorbing light at 337 nm and emitting at 620 nm. Thus, the emission maximum of Europium cryptate (Eu3+K) overlaps the absorption maximum of XL665. Europium cryptate (Eu3+K) has a large temporal shift; the time between absorption and emission of a photon is about 1 millisecond. This is advantageous
25 because most background fluorescence signals in biological samples are short-lived. Thus the use of a fluorescent reagent such as europium cryptate, with a long fluorescent lifetime, permits time-resolved detection resulting in the reduction of background interference.

 The spectral and temporal properties of europium cryptate (Eu3+K) result in essentially no fluorescence background and thus
30 assays using this fluorescent reagent can be carried out in a "mix and read" mode, greatly facilitating its use as a high throughput screening tool. For the embodiment using Europium cryptate (Eu3+K) and XL665, the measuring instrument irradiates the sample at 337 nm and
35 measures the fluorescence output at two wavelengths, 620 nm (B counts, europium fluorescence) and 665 nm (A counts, XL665 fluorescence).

The extent of fluorescent resonance energy transfer is measured as the ratio between these two values. Typically this ratio is multiplied by 10,000 to give whole numbers.

Other FRET donor-acceptor pairs are suitable for the practice of the present invention. For example, the following donor-acceptor pairs can be used: dansyl/fluorescein; fluorescein/rhodamine; tryptophan/aminocoumarin.

The present invention provides a nuclear receptor or ligand binding domain thereof labeled with a fluorescent reagent for use in the above-described methods of identifying agonists and antagonists of nuclear receptors. The present invention also provides CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a fluorescent reagent.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is selected from the group consisting of PPAR α , PPAR γ , PPAR δ , a ligand binding domain of PPAR α , PPAR γ , or PPAR δ , and amino acid residues 176-478 of human PPAR γ 1 and the fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

In a particular embodiment, CBP, p300, or other nuclear receptor co-activator is labeled with a fluorescent reagent selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

Cloning, expression, and purification of human CBP and PPAR proteins

To test whether human CBP can interact with PPARs in an agonist-dependent manner, we cloned the human cDNA fragments encoding the NH₂-terminal 1-113 amino acids (hCBP1-113) and 1-453 amino acids (hCBP1-453) of human CBP by the polymerase chain reaction (PCR). The DNA and amino acid sequences of human CBP are

disclosed in Borrow et al., 1996, Nature Genet. 14:33-41 and in GenBank, accession no. U47741.

The primers used for hCBP1-113 were:

5'-ACTCGGATCCAAGCCATGGCTGAGAACTTGCTGGACGG-3'

5 (SEQ.ID.NO.:9) and

5'-CACAAAGCTTAGGCCATGTTAGCACTGTTCCGG-3' (SEQ.ID.NO.:10).

These primers were expected to amplify a 0.9 kb DNA fragment.

The primers for hCBP1-453 were:

10 5'-ACTCGGATCCAAGCCATGGCTGAGAACTTGCTGGACGG-3'
(SEQ.ID.NO.:9) and

5'CTCAGTCGACTTATTGAATTCCACTAGCTGGAGATCC-3'
(SEQ.ID.NO.:11).

These primers were expected to amplify a 1.5 kb DNA fragment..

15 The template for the PCR reaction was a human fetal brain cDNA library (Stratagene, Catalogue #IS 937227). Of course, any human cDNA library from a tissue expressing CBP could have been used. The PCR amplified 0.9 kb and 1.5 kb DNA fragments which were digested with restriction endonucleases and ligated into pBluescript II
20 vector. DNA sequencing analysis confirmed that the amplified fragments were identical to the corresponding published nucleic acid sequences of human CBP.

Based on the publicly available sequences for human CBP cited above, other primers could be readily identified and prepared by
25 those skilled in the art in order to amplify and clone other portions of cDNA encoding human CBP from appropriate cDNA libraries. Once such portions of human CBP are produced, they could be used in the methods of the present invention in a manner similar to that described herein for hCBP1-113 and hCBP1-453. The amino acid sequence of
30 human CBP is shown in Figure 7A; the nucleic acid sequence of the cDNA encoding human CBP is shown in Figure 7B.

To express the polypeptides encoded by the PCR fragments, vectors encoding fusion proteins of the polypeptides and glutathione S-transferase (GST) were constructed and expressed in *E. coli*. The PCR
35 fragments were subcloned into the expression vector pGEX (Pharmacia Biotech) to generate pGEXhCBP1-113 and pGEXhCBP1-453.

pGEXhCBP1-113 and pGEXhCBP1-453 were transfected into the DH5 α strain of *E. coli* (GIBCO BRL) and the bacteria hosting either pGEXhCBP1-113 or pGEXhCBP1-453 were cultured in LB medium (GIBCO BRL) to a density of OD₆₀₀ = 0.7-1.0 and induced for
5 overexpression of the GST-CBP fusion proteins by addition of IPTG (isopropylthio- β -galactoside) to a final concentration of 0.2 mM. The IPTG induced cultures were further grown at room temperature for 2-5 hrs. The cells were harvested by centrifugation for 10 min at 5000g. The cell pellet was used for GST-CBP fusion protein purification by following
10 the procedure from Pharmacia Biotech using Glutathione Sepharose beads. hCBP1-113 and hCBP1-453 proteins were generated by cleaving the corresponding GST fusion proteins with thrombin. SDS-polyacrylamide gel electrophoresis analysis showed that the preparation from pGEXhCBP1-113 gave two polypeptide bands, with apparent
15 molecular weight of 12 kd and 10 kd. The 12 kd band is the expected size of hCBP1-113 and the 10 kd band is most likely a premature translational termination product. The preparation from pGEXhCBP1-450 gave a single band with the expected size, 50 kd.

cDNAs encoding full-length PPAR α and PPAR γ 1 were
20 subcloned into pGEX vectors for the production of GST-PPAR α and GST-PPAR γ 1 fusion proteins in *E. coli*. PPAR γ 1 was cloned from a human fat cell cDNA library (see Elbrecht et al., 1996, Biochem. Biophys. Res. Comm. 224:431-437). A cDNA encoding the human PPAR γ 1 ligand binding domain (PPAR γ 1LBD; amino acids 176-478 of PPAR γ 1) was
25 subcloned from a modified pSG5 vector as a Xho I (site located in the N-terminus of the LBD)/ Xba I (site located in the pSG5 vector) fragment. The Xba I site was blunt-ended with T4 DNA polymerase. The 1.1 kb fragment containing the LBD was purified from an agarose gel and ligated into pGEX-KG (see Guan & Dixon, 1991, Anal. Biochem. 192:262-
30 267) that had been digested with Xho I and Hind III (the Hind III site had been blunt-ended with T4 DNA polymerase). This construct was used for the production of GST-hPPAR γ 1LBD and hPPAR γ 1LBD (the ligand binding domain cleaved free of GST). The overexpression and purification of PPAR α , PPAR γ 1, and PPAR γ 1LBD were as described
35 above for CBP.

The DNA and amino acid sequences of human PPAR α are disclosed in Schmidt et al., 1992, Mol. Endocrinol. 6:1634-1641 and in GenBank, accession no. L07592. See Figure 8A and 8B.

5 The DNA and amino acid sequences of human PPAR γ 1 are disclosed in Greene et al., 1995, Gene Expr. 4:281-299; Qi et al., 1995, Mol. Cell. Biol. 15:1817-1825; Elbrecht et al., 1996, Biochem. Biophys. Res. Comm. 224:431-437; and in GenBank, accession no. L40904. See Figure 9A and 9B. Human PPAR γ 2 contains the same amino acid sequence as human PPAR γ 1 except for an amino terminal addition of 24 amino acids
10 (see Elbrecht et al., 1996, Biochem. Biophys. Res. Comm. 224:431-437). Thus, the amino acid sequence of the ligand binding domain of human PPAR γ 2 is the same as the amino acid sequence of the ligand binding domain of human PPAR γ 1, although the numbering of the amino acids differs (176-478 for human PPAR γ 1 and 200-502 for human PPAR γ 2).

15 The DNA and amino acid sequences of human PPAR δ are disclosed in Sher et al., 1993, Biochemistry 32:5598-5604 and in GenBank, accession no. L02932. See Figure 10A-C.

EXAMPLE 2

20 Interaction between PPARs and hCBP fragments

Experiments were first conducted using hCBP1-113 and hPPAR γ 1LBD. Purified hPPAR γ 1LBD was biotinylated with Sulfo-NHS-LC-Biotin (PIERCE) to a biotin:hPPAR γ 1LBD ratio of 3:1 according to the procedure provided by PIERCE. Purified hCBP1-113 was directly labeled
25 with europium cryptate (Eu3+K) by the method illustrated in Figure 1. Biotin-labeled hPPAR γ 1LBD, Eu3+K-labeled hCBP1-113, and streptavidin-labeled XL665 (SA-XL665; from PACKARD) were incubated together in the presence or absence of 1 μ M of known PPAR γ agonist (BRL49653 or AD5075).

30 Thus, this experimental format made use of the fluorescent reagent pair europium cryptate (Eu3+K), which acted as donor, and XL665, which acted as acceptor. hCBP1-113 was directly labeled with europium cryptate (Eu3+K); hPPAR γ 1LBD was indirectly labeled with XL665 by means of a biotin-streptavidin link. The emission maximum

of europium cryptate (Eu3+K) overlaps with the absorption maximum of XL665. Therefore, when europium cryptate (Eu3+K) and XL665 are in close proximity, and the sample is illuminated with light at 337 nm (the absorption maximum of europium cryptate (Eu3+K)), FRET can occur
5 between europium cryptate (Eu3+K) and XL665. This FRET manifests itself as increased emission at 665 nm by XL665. Figure 2 shows a schematic of the format used in this experiment (experiment 1 of Table 1). When agonist is bound to hPPAR γ 1LBD, a specific interaction occurs between hPPAR γ 1LBD and hCBP1-113, thus bringing europium cryptate
10 (Eu3+K) and XL665 into close enough proximity for FRET to occur. In the absence of agonist, no interaction occurs between hPPAR γ 1LBD and hCBP1-113 and thus europium cryptate (Eu3+K) and XL665 are not brought into close proximity and no FRET occurs. When FRET occurs, the amount of light given off by the sample at the emission maximum of
15 XL665 (665 nm) is increased relative to the amount of light given off by the sample at the emission maximum of europium cryptate (Eu3+K) (620 nm). Therefore, measuring the ratio of emission at 665 nm to 620 nm in the presence and the absence of a substance suspected of being an agonist allows for the determination of whether that substance actually
20 is an agonist. If the substance is an agonist, an increase in the ratio of emission at 665 nm to 620 nm in the presence of the substance will be observed.

Reactions were carried out in microtiter plates. Reaction conditions were: appropriate volume (total 250 μ l) of the reaction buffer
25 (either PBS or HEPES, see below, containing 500 mM KF, 0.1% bovine serum albumin, BSA) was added to each well, followed by addition of ligands (BRL49653 or AD5075 at a final concentration of 1 μ M and 0.1% dimethylsulfoxide (DMSO) or vehicle control (0.1% DMSO), Eu3+K labeled hCBP (100 nM), biotin-hPPAR γ 1LBD (100 nM), and streptavidin-
30 labeled XL665 (100 nM) to appropriate wells. After mixing, 200 μ l of reaction mixture was transferred to a new well. The plate was either directly measured for fluorescence resonance energy transfer (FRET) or covered with sealing tape (PACKARD) to avoid evaporation and incubated at room temperature for up to 24 hrs before measuring FRET.

35 The results of this experiment and others described below yielded ratio values as follows:

Table 1

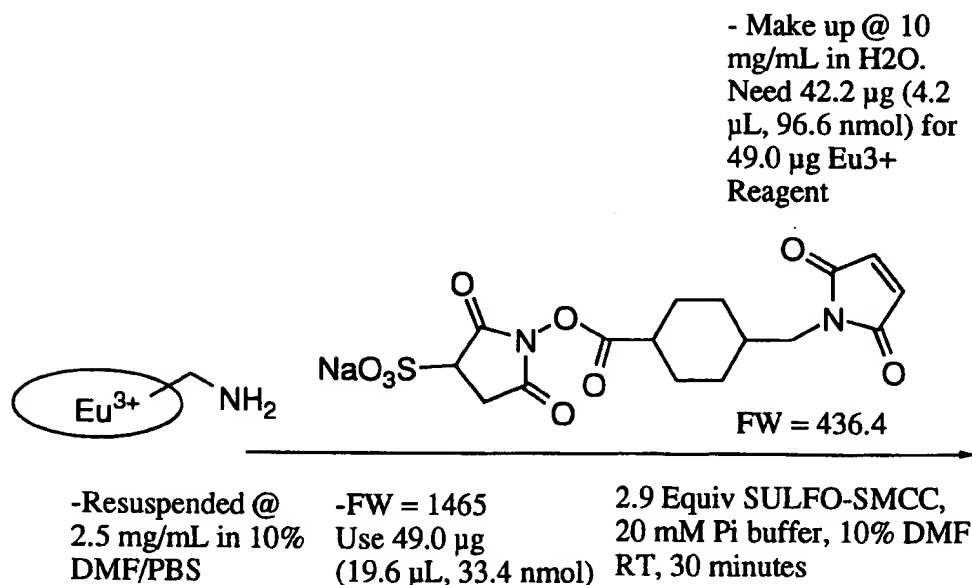
Experiment	Buffer	Emission ratio with AD5075	Emission ratio with vehicle
1	PBS	1134	1074
2	HEPES + 0.05% NP40	967	617
3	HEPES + 0.05% NP40	1078	536
4	HEPES + 0.05% CHAPS	1883	487

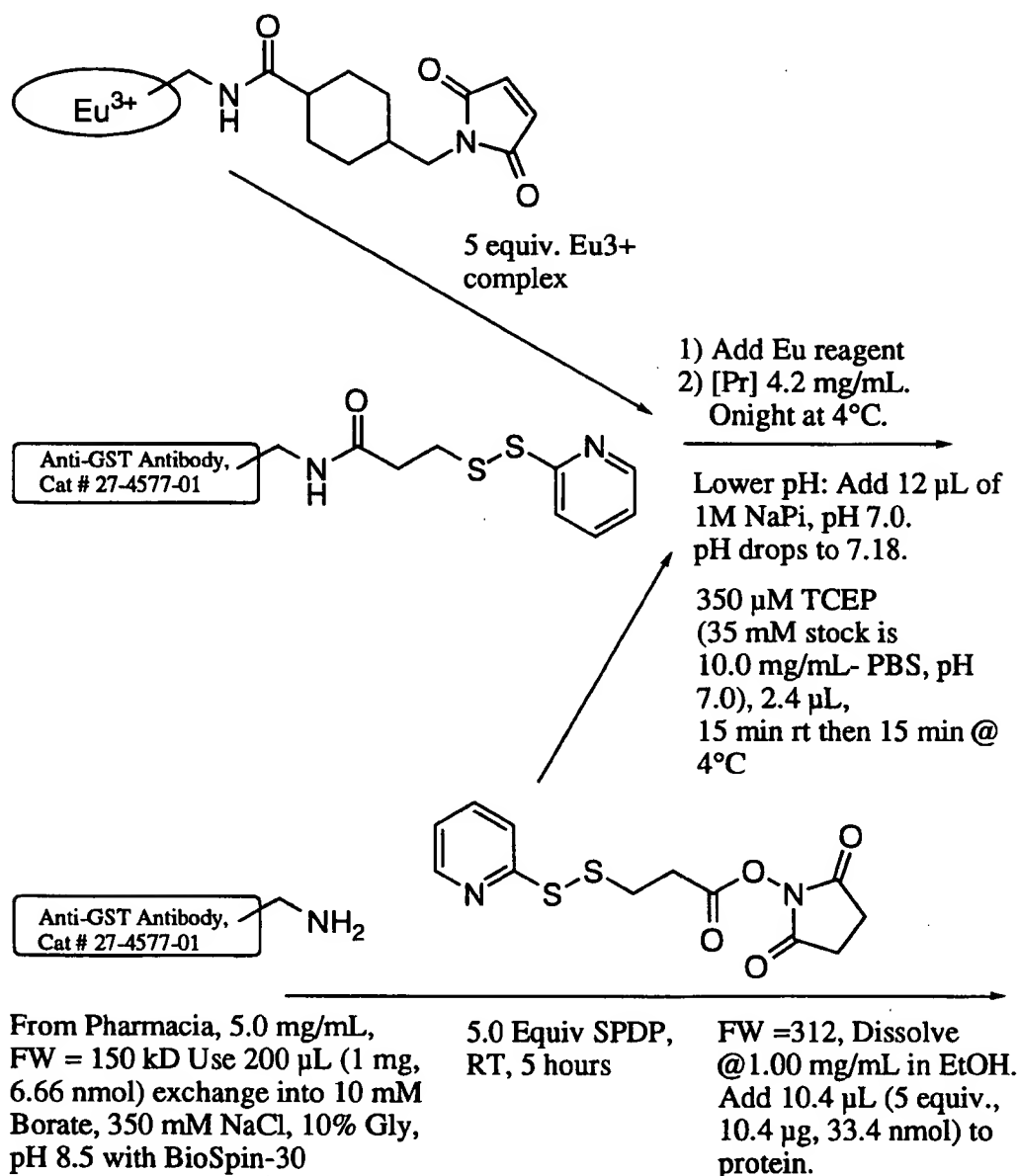
Experiment 1 of Table 1 was carried out using PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, pH 7.4). The greater emission ratio observed in the presence of AD5075 demonstrated that a specific interaction between hCBP1-113 and hPPAR γ 1LBD

5 occurred in the presence of the agonist AD5075. Although it was clear that FRET was occurring, the signal-noise ratio was small. In experiment 2 of Table 1, HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid, 100 mM, pH 7.0) containing 0.05% NP40 (Nonidet P-40) was used instead of PBS and an improved signal-noise ratio was
10 obtained.

In order to get an even better signal-noise ratio, the above-described format was modified slightly for experiment 3. In experiment 3, SA-XL665 (500 nM), biotin-labeled hPPAR γ 1LBD (100 nM), GST-hCBP1-113, and Eu3+K labeled anti-GST antibody (2.5 μ l) were incubated
15 in the presence or absence of AD5075 (1 μ M) in HEPES buffer containing 0.05% NP40. A two-fold signal- noise ratio was obtained. Figure 3 shows a schematic of the format used in experiment 3.

The anti-GST antibody was a goat antibody to GST from Pharmacia (catalogue number 27-4577-01) that was labeled with Eu3+K
20 according to the procedure summarized below.





To further improve the signal to noise ratio, a series of experiments were conducted. Experiment 4 of Table 1 exemplifies results obtained from those efforts. cDNA encoding a longer fragment of hCBP was cloned and expressed to get hCBP1-453. hCBP1-453 was biotinylated. Biotin-labeled hCBP1-453 (25 nM), SA-XL665 (100 nM), GST-hPPAR γ 1LBD (1 nM), and Eu³⁺-K-labeled anti-GST antibody (2 nM) were mixed together in the presence or absence of 1 µM AD5075. The detergent was changed from 0.05% NP40 to 0.5% CHAPS (3-[[3-cholamidopropyl]dimethyl-ammonio]-1-propanesulfonate). A three- to

four-fold signal-noise ratio was obtained. Figure 4 shows the strategy used for experiment 4 and similar experiments.

The correlation between results from the above-described assays and previously reported results from *in vitro* binding and transcriptional activation assays of selected antidiabetic insulin sensitizers that are known to be PPAR γ agonists (Elbrecht et al., 1996, Biochem Biophys Res Comm 224:431-437) was analyzed by titrating those known PPAR γ agonists in the assays described above and comparing EC50s so obtained with previously described values for potency in binding or transcriptional activation assays for the known agonists. The results are shown in Figure 5. From Figure 5, the following EC50s can be derived:

AD5075 = 8 nM

BRL49653 = 53 nM

Troglitazone = 646 nM

Pioglitazone = 890 nM.

These EC50s generated in the above-described assays are in close agreement with those generated by *in vitro* binding and transcriptional activation studies (Elbrecht et al., 1996, Biochem Biophys Res Comm 224:431-437).

The above-described assay can also be used to characterize the interaction between nuclear receptors with co-activators as, e.g., by determining the binding constant for that interaction. Figure 6 shows an example of such an application. Saturating amounts of PPAR γ agonist (10 μ M BRL49653) were used. Increasing concentrations of non-biotinylated hCBP1-453 were used to titrate away biotin-hCBP-PPAR γ 1LBD complex and decrease the fluorescence energy transfer. A Kd of 300 nM for the interaction between hCBP1-453 and PPAR γ 1LBD can be derived from the results illustrated in Figure 6 and this Kd (300 nM) is a measurement of the affinity between CBP and PPAR γ .

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED:

1. A method of identifying an agonist of a nuclear receptor that comprises providing:

- 5 (a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
- (b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent; and
- (c) a substance suspected of being an agonist of the nuclear receptor;
- 10 under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, will occur; and
- (d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;
- 15 where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

2. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, LXR, and FXR.

20

3. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a full-length nuclear receptor, a ligand binding domain of a nuclear receptor, and an AF-2 site of a nuclear receptor.

25

4. The method of claim 1 where the nuclear receptor or ligand binding domain thereof comprises an AF-2 site of a nuclear receptor.

30

5. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a

35

full-length PPAR, a ligand binding domain of a PPAR, and amino acid residues 176-478 of human PPAR γ 1.

5 6. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of PPAR α , PPAR γ 1, PPAR γ 2, and PPAR δ .

10 7. The method of claim 1 where the nuclear receptor or ligand binding domain thereof comprises a ligand binding domain selected from the group consisting of amino acids 143-462 of human RAR α , amino acids 122-410 of rat T3R α 1, amino acids 227-463 of mouse RXR γ , and amino acids 251-595 of human ER.

15 8. The method of claim 1 where CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof is selected from the group consisting of full-length human CBP, full-length mouse CBP, amino acid residues 1-113 of human CBP, and amino acid residues 1-453 of human CBP.

20 9. The method of claim 1 where the first fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

25 10. The method of claim 1 where the second fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

30 11. A method of identifying an agonist of a nuclear receptor that comprises providing:
 (a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
 (b) a binding portion of a nuclear receptor co-activator, where the binding portion contains the amino acid sequence LXXLL, and where the binding portion is labeled with a second fluorescent
35 reagent; and

(c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the binding portion of the nuclear receptor co-activator will take place; and

(d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

12. The method of claim 11 where the binding portion of a nuclear receptor co-activator is selected from the group consisting of human RIP-140, human SRC-1, mouse TIF-2, human or mouse CBP, human or mouse p300, mouse TIF-1, and human TRIP proteins.

13. A method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) a polypeptide containing the amino acid sequence LXXLL where the polypeptide is labeled with a second fluorescent reagent; and

(c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the polypeptide will take place; and

(d) measuring fluorescent resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

14. A method for identifying an antagonist of a nuclear receptor that comprises providing:

- (a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
- (b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent;
- 5 (c) an agonist of the nuclear receptor; and
- (d) a substance suspected of being an antagonist of the nuclear receptor;

under conditions such that, in the absence of the substance, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding
10 portion thereof will occur; and

- (e) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents when the substance is present and measuring FRET between the first and second
15 fluorescent reagents when the substance is absent;

where the a decrease in FRET when the substance is present indicates that the substance is an antagonist of the nuclear receptor.

20 15. The method of claim 14 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, LXR, and FXR.

25 16. The method of claim 14 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a full-length nuclear receptor, a ligand binding domain of a nuclear receptor, and an AF-2 site of a nuclear receptor.

30 17. The method of claim 14 where the nuclear receptor or ligand binding domain thereof comprises an AF-2 site of a nuclear receptor.

35 18. The method of claim 14 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a

full-length PPAR, a ligand binding domain of a PPAR, and amino acid residues 176-478 of human PPAR γ 1.

19. The method of claim 14 where the nuclear receptor or
5 ligand binding domain thereof is selected from the group consisting of PPAR α , PPAR γ 1, PPAR γ 2, and PPAR δ .

20. The method of claim 14 where the nuclear receptor or
ligand binding domain thereof comprises a ligand binding domain
10 selected from the group consisting of amino acids 143-462 of human RAR α , amino acids 122-410 of rat T3R α 1, amino acids 227-463 of mouse RXR γ , and amino acids 251-595 of human ER.

21. The method of claim 14 where CBP, p300, or other
15 nuclear receptor co-activator, or a binding portion thereof is selected from the group consisting of full-length CBP, amino acid residues 1-113 of human CBP, and amino acid residues 1-453 of human CBP.

22. The method of claim 14 where the first fluorescent
20 reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

23. The method of claim 14 where the second fluorescent
25 reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

24. A nuclear receptor or ligand binding domain thereof
labeled with a fluorescent reagent.

25. The nuclear receptor or ligand binding domain
30 thereof of claim 24 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of PPAR α , PPAR γ 1, PPAR γ 2, PPAR δ , a ligand binding domain of PPAR α , PPAR γ 1, PPAR γ 2, or PPAR δ , and amino acid residues 176-478 of human PPAR γ 1 and the
35 fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

26. CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a fluorescent reagent.

- 5 27. The CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, of claim 26 where the fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu³⁺+K).

1/11

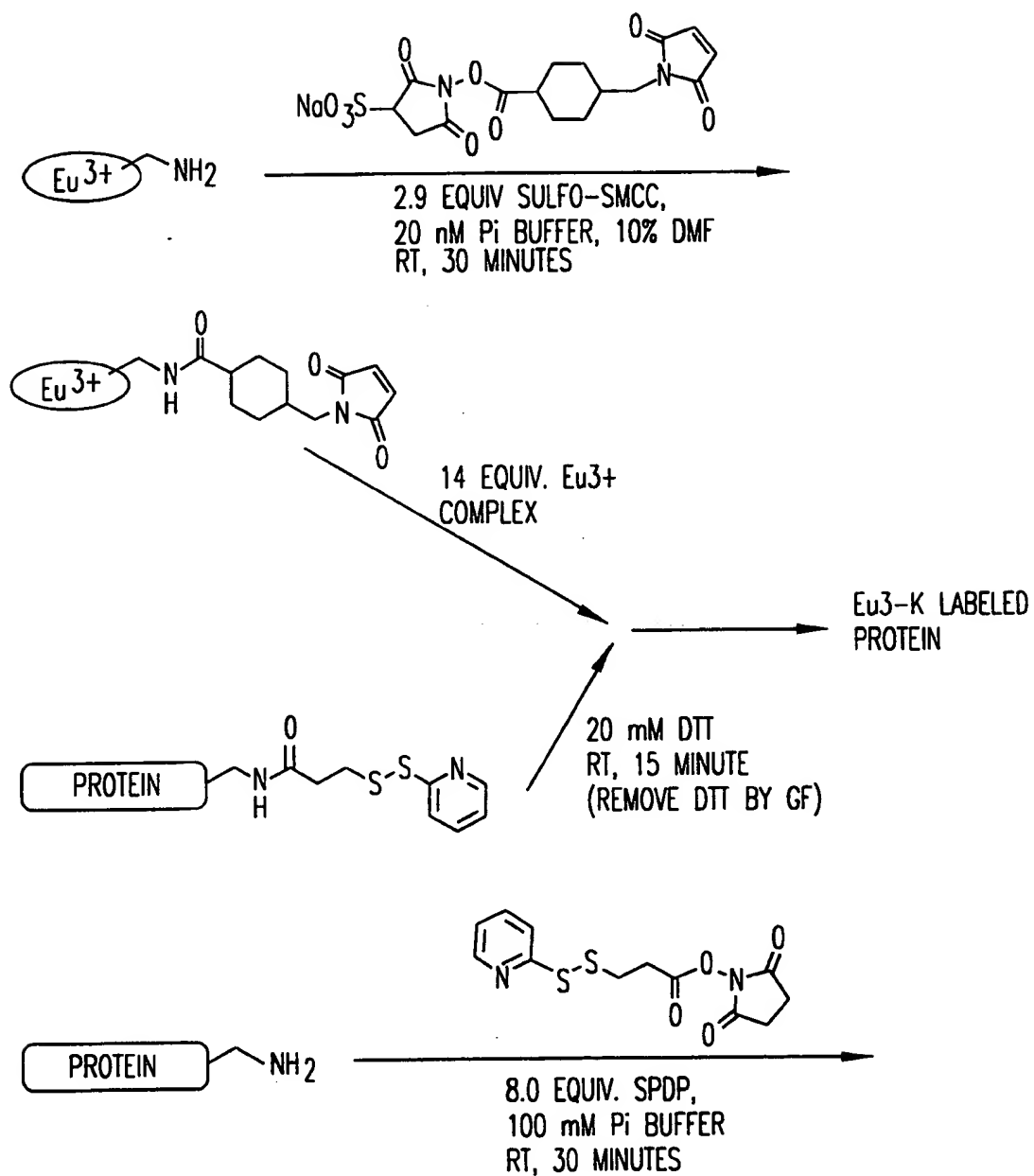


FIG.1

2/11

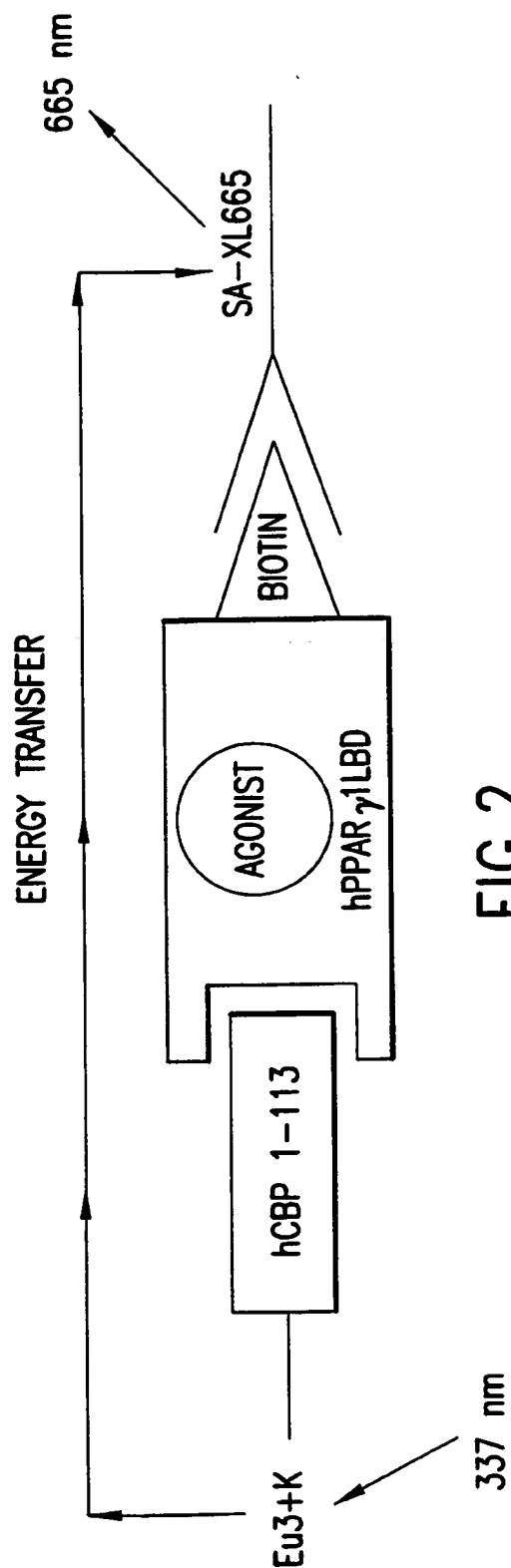


FIG. 2

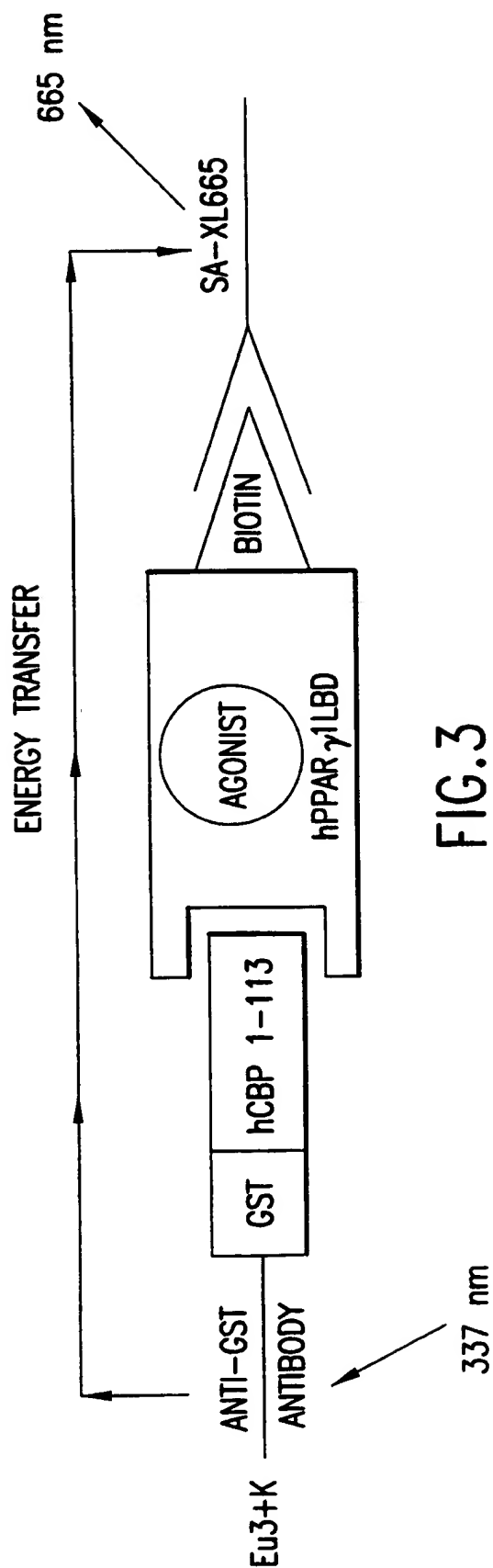


FIG. 3

3/11

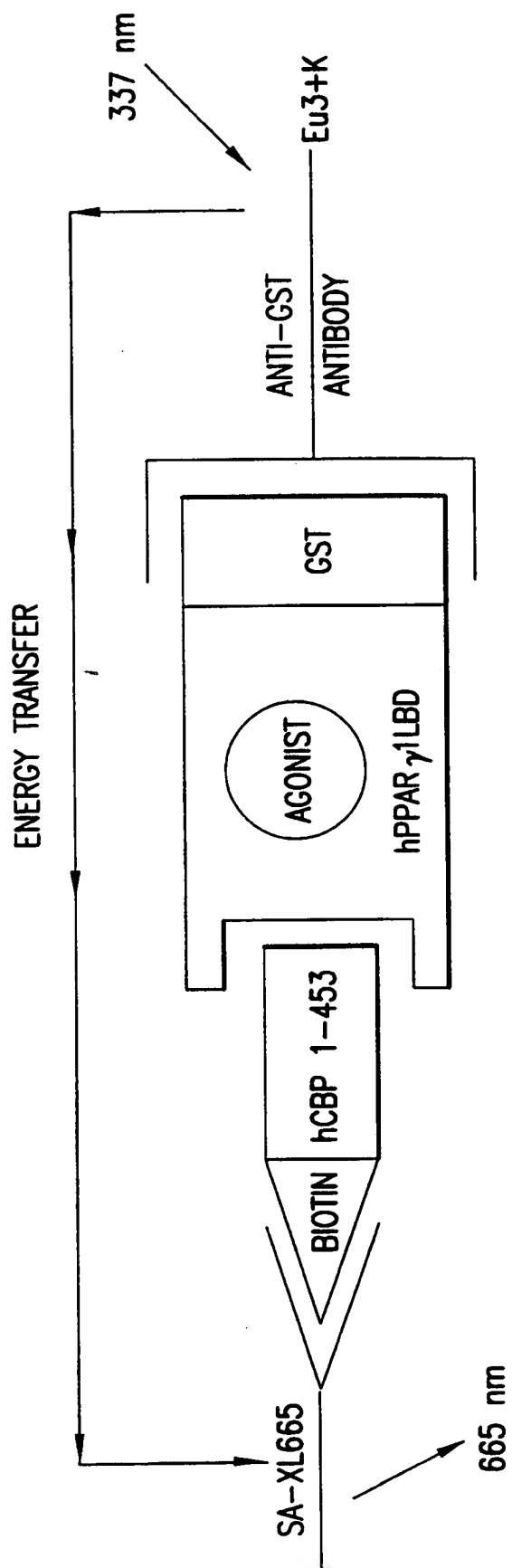


FIG. 4

4/11

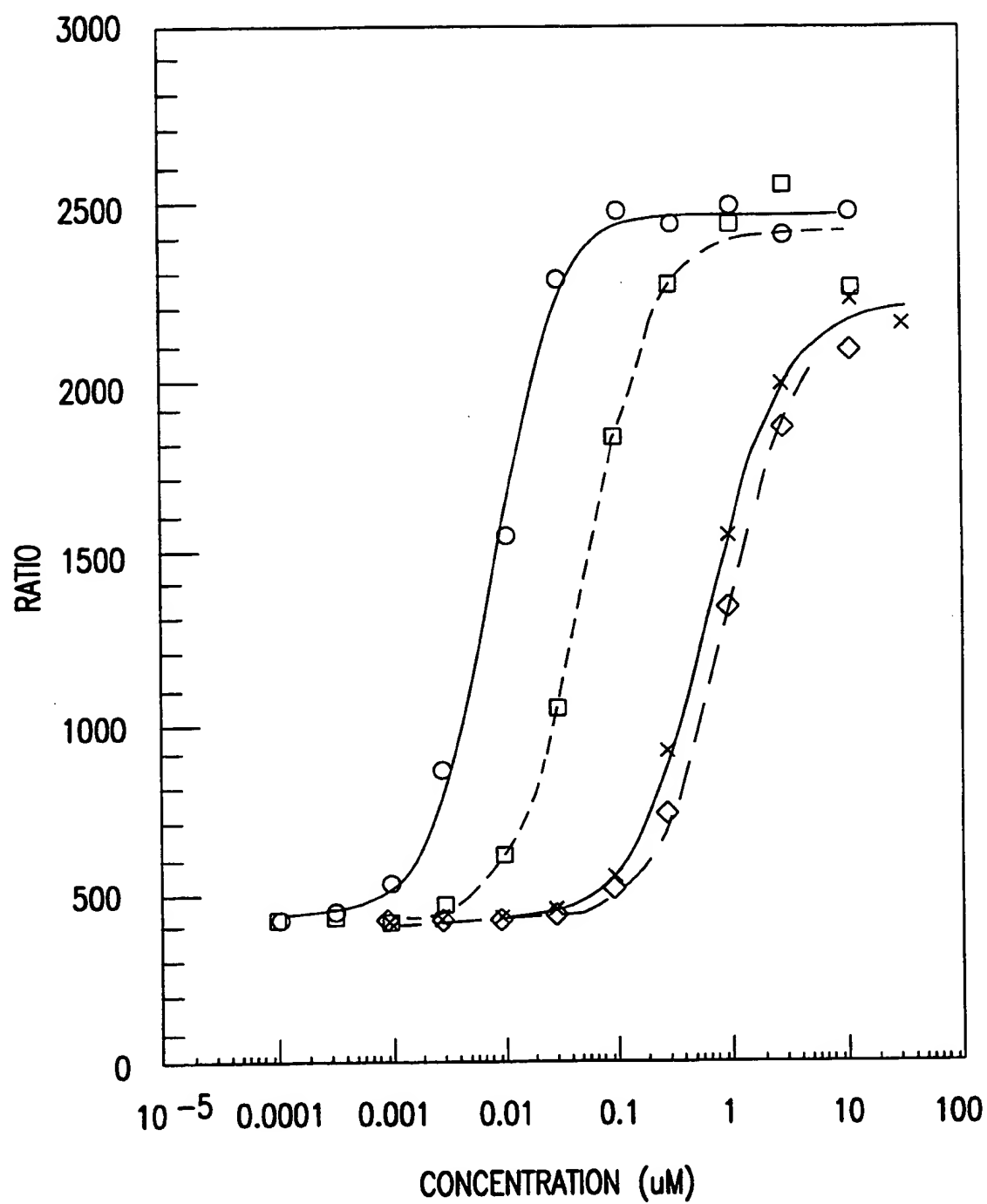


FIG.5

5/11

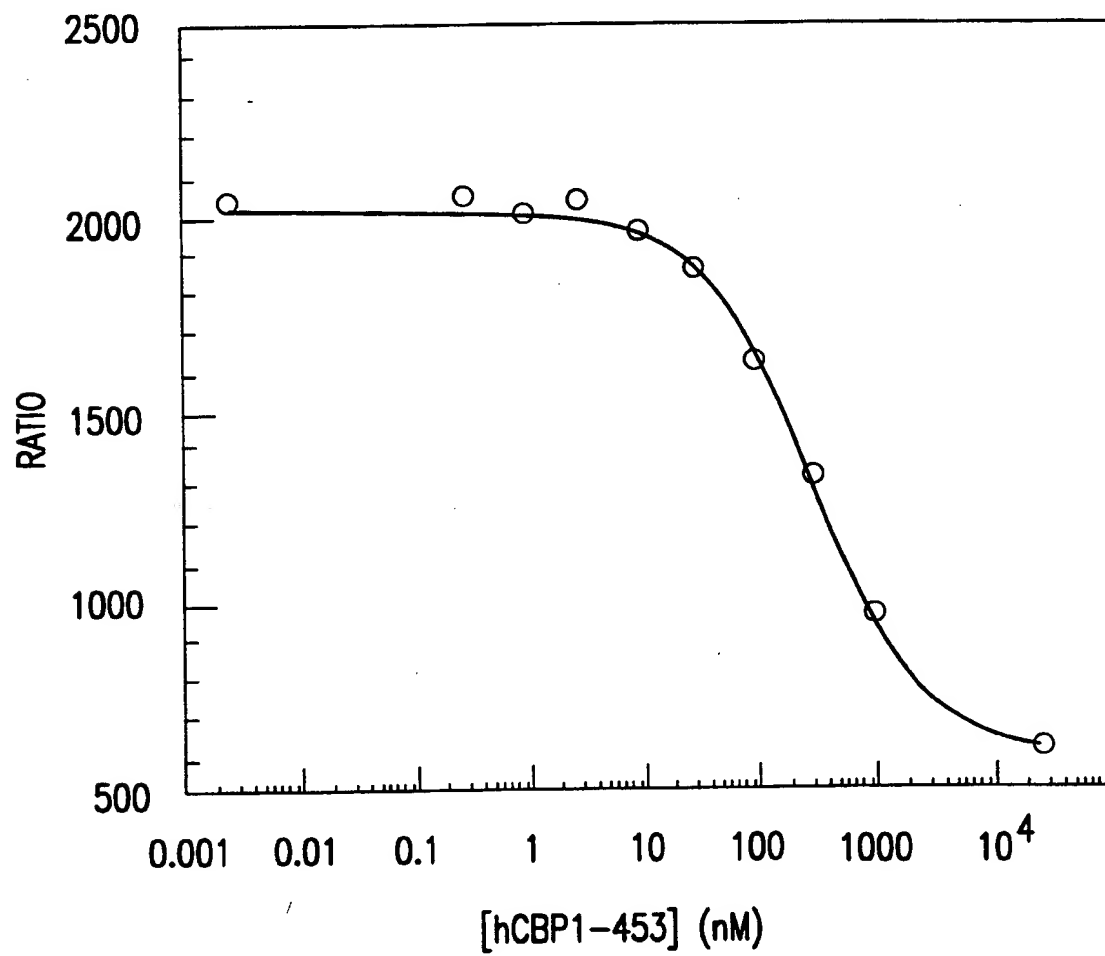


FIG.6

6/11

1 MAENLLDGPPNPKRAKLSSPGFSANDSTDFGSLFDLENDLPDELPNGGELGLLNSGNLV
 61 PDAASKHKQLSELLRGGSGSSINPGIGNVSASSPVQOGLGGQAQGPNSANMASLSAMGK
 121 SPLSQGDSSAPSLPKQAASTSGPTPAASQALNPQAQKQVGLATSSPATSTGPGICMNAN
 181 FNQTHPGLLNSNSGHSLINQASQGQAQVMNGSLGAAGRGRGAGMPYPTPAMQGASSSVLA
 241 ETLTQVSPQMTGHAGLNTAQAGGMAKMGITGNTSPFGQPFQAGGQPMGATGVNPQLASK
 301 QSMVNSLPTFPTDIKNTSVTNVPNMSQMOTSVGIVPTQAIATGPTADPEKRKLIQQQLVL
 361 LLHAHKCQRREQANGEVRACSLPHCRTMKNVLNHMTHCQAGKACQ

FIG.7A

1 cgagccccga cccccgtccg ggccctcgcc ggccgcgccg cccgtgccccg gggctgtttt
 61 cccgagcagg tgaaaatggc tgagaacttg ctggacggac cgcccaaccc caaaagagcc
 121 aaactcagct cgcccgtttt ctcggcgaat gacagcacag attttggatc attgtttgac
 181 ttggaaaatg atcttctga tgagctgata cccaatggag gagaattagg ccttttaaac
 241 agtgggaacc ttgttcaga tgctgcttcc aaacataaac aactgtcggg gcttctacga
 301 ggaggcagcg gctctagtat caaccagga ataggaaatg tgagcgccag cagccccgtg
 361 cagcagggcc tgggtggcca ggctcaaggg cagccgaaca gtgctaacat ggccagcctc
 421 agtgccatgg gcaagagccc tctgagccag ggagattctt cagccccag cctgcctaaa
 481 caggcagcca gcacctctgg gccaccccc gctgcctccc aagcactgaa tccgcaagca
 541 caaaagcaag tggggctggc gactagcagc cctgccacgt cacagactgg acctggtatc
 601 tgcatgaatg ctaactttaa ccagaccac ccaggcctcc tcaatagtaa ctctggccat
 661 agcttaatta atcaggcttc acaagggcag gcgcaagtca tgaatggatc tcttggggct
 721 gctggcagag gaaggggagc tggaatgccg taccctactc cagccatgca gggcgccctg
 781 agcagcgtgc tggctgagac cctaacgcag gtttccccgc aaatgactgg tcacgcggga
 841 ctgaacaccg cacaggcagg aggcattggc aagatgggaa taactgggaa cacaagtcca
 901 tttggacagc cctttagtca agctggaggg cagccaatgg gagccactgg agtgaacccc
 961 cagttagcca gcaaacagag catggtcaac agtttgccca ccttcctac agatatcaag
 1021 aatacttcag tcaccaacgt gccaaatatg tctcagatgc aaacatcagt ggggaattgta
 1081 cccacacaag caattgcaac aggccccact gcagatcctg aaaaacgcaa actgatacag
 1141 cagcagctgg ttctactgct tcatgctcat aagtgtcaga gacgagagca agcaaacgga
 1201 gaggttcggg cctgctcgct cccgcattgt cgaacatga aaaacgtttt gaatcacatg
 1261 acgcattgtc aggctgggaa agcctgccaa

FIG.7B

7/11

1 MVDTESPLCPLSPLEAGDLESPLSEEFLOQEMGNIQEISQSIGEDSSGSFGFTEYQYLGSC
 61 PGSDGSVITDTLSPASSPSSVTYPVVPGSVDESPSGALNIECRICGDKASGYHYGVHACE
 121 GCKGFFRRTIRLKLVDKCDRSCKIQKKNRNCQYCRFHKCLSVGMSHNAIRFGRMPRSE
 181 KAKLKAEILTCEHDIEDSETADLKSLAKRIYEAYLKNFNMNKVKARVILSGKASNNPPFV
 241 IHDMETLCMAEKTIVAKLVANGIQNKEVEVRIFHCCQCTSVETVTELTEFAKAIPAFANL
 301 DLNDQVTLTKYGVYEAIFAMLSVMNKDGMVLVAYGNGITREFLKSURKPFCDIMEPKFD
 361 FAMKFNALELDDSDISLFVAAIICCGDRPGLLNVGHIEMQEGIVHVURLHLQSNHPDDI
 421 FLPKLLQKMADLRQLVTEHAQLVQIIKKTESDAALHPLLQEIYRDMY

FIG.8A

1 ggcccaggct gaagctcagg gccctgtctg ctctgtggac tcaacagttt gtggcaagac
 61 aagctcagaa ctgagaagct gtcaccacag ttctggaggc tgggaagttc aagatcaaag
 121 tgccagcaga ttcagtgtca tgtgaggacg tgcttcctgc ttcatagata agagtagctt
 181 ggagctcggc ggcacaacca gcaccatctg gtcgcgatgg tggacacgga aagcccactc
 241 tgccccctct cccactcga ggccggcgat ctagagagcc cgttatctga agagttcctg
 301 caagaaatgg gaaacatcca agagatttcg caatccatcg gcgaggatag ttctggaagc
 361 tttggcttta cgaataacca gtatttagga agctgtcctg gtcagatgg ctcggtcac
 421 acggacacgc tttcaccagc ttcgagcccc tctcgggtga cttatcctgt ggtccccggc
 481 agcgtggacg agtctcccag tggagcattg aacatcgaat gtagaatctg cggggacaag
 541 gcctcaggct atcattacgg agtccacgcg tgtgaaggct gcaagggctt ctttcggcga
 601 acgattcgac tcaagctggg gtatgacaag tgcgaccgca gctgcaagat ccagaaaaag
 661 aacagaaaca aatgccagta ttgtcgattt cacaagtgcc tttctgtcgg gatgtcacac
 721 aacgcgattc gttttggacg aatgccaaga tctgagaaag caaaactgaa agcagaaatt
 781 cttacctgtg aacatgacat agaagattct gaaactgcag atctcaaate tctggccaag
 841 agaatctacg aggcctactt gaagaacttc aacatgaaca aggtcaaagc ccgggtcatc
 901 ctctcaggaa aggccagtaa caatccacct tttgtcatac atgatattga gacactgtgt
 961 atggctgaga agacgctggg ggccaagctg gtggccaatg gcatccagaa caaggagggtg
 1021 gaggtccgca tctttcactg ctgccagtgc acgtcagtgg agaccgtcac ggagctcacg
 1081 gaattcgcca aggccatccc agcgttcgca aacttgacc tgaacgatca agtgacattg
 1141 ctaaaatacg gagtttatga ggccatattc gccatgctgt cttctgtgat gaacaaagac
 1201 gggatgctgg tagcgtatgg aaatggggtt ataactcgtg aattcctaaa aagcctaagg
 1261 aaaccgttct gtgatcat ggaacccaag tttgattttg ccatgaagtt caatgcactg
 1321 gaactggatg acagtgatat ctcccttttt gtggctgcta tcatttgctg tggagatcgt
 1381 cctggccttc taaacgtagg acacattgaa aaaatgcagg agggatttgt acatgtgctc
 1441 agactccacc tgcagagcaa ccacccggac gatattcttc tcttcccaaa acttcttcaa
 1501 aaaatggcag acctccggca gctggtagcg gagcatgccc agctgggtgca gatcatcaag
 1561 aagacggagt cggatgctgc gctgcacccg ctactgcagg agatctacag ggacatgtac
 1621 tgagttcctt cagatcagcc acaccttttc caggagtctt gaagctgaca gcactacaaa
 1681 ggagacgggg gagcagcacg attttgcaca aatatccacc actttaacct tagagcttgg
 1741 acagtctgag ctgtaggtaa ccggcatatt attccatata tttgttttaa ccagtacttc
 1801 taagagcata gaactcaaat gctgggggag gtggctaata tcaggactgg gaag

FIG.8B

8/11

1 MTMVDTEIAFWPTNFGISSVDLSVMEDHSHSFDIKPFTTVDFSSISTPHYEDIPFTRTDP
 61 VVADYKYDLKLQEYQSAIKVEPASPPYYSEKTQLYNKPHEEPSNSLMAIECRVCGDKASG
 121 FHYGVHACEGCKGFFRRTIRLKLIDRCDLNCRHKKSRNKCQYCRFQKCLAVGMSHNAI
 181 RFGRIAQAEKEKLLAEISSDIDQLNPESADLRQALAKHLYDSYIKSFPLTKAKARAILTG
 241 KTTDKSPFVIYDMNSLMMGEDKIKFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEY
 301 AKSIPGFVNLDLNDQVTLLKYGVHEIIYTMLASLMNKDGVLISEGQGFMTREFLKSRLKP
 361 FGDFMEPKFEFAVKFNALELDDSDLAIFIAVIIISGDRPGLLNPKPIEDIQDNLLQALEL
 421 QLKLNHPESSQUAKLLQKMTDLRQIVTEHVQLLQVIKKTETDMSLHPLLQEIKDLY

FIG.9A

1 ccgaccttac cccaggcggc cttgacgttg gtcttgctcg caggagacag caccatggtg
 61 ggttctctct gagtctggga attcccgagc ccgagccgca gccgccgcct ggggggcttg
 121 ggtcggcctc gaggacaccg gagagggcg ccacgccgcc gtggccgcag aaatgaccat
 181 ggttgacaca gagatcgcat tctggccac caactttggg atcagctccg tggatctctc
 241 cgtaatggaa gaccactccc actcctttga tatcaagccc ttcactactg ttgacttctc
 301 cagcatttct actccacatt acgaagacat tccattcaca agaacagatc cagtggttgc
 361 agattacaag tatgacctga aacttcaaga gtaccaaagt gcaatcaaag tggagcctgc
 421 atctccacct tattattctg agaagactca gctctacaat aagcctcatg aagagccttc
 481 caactccctc atggcaattg aatgtcgtgt ctgtggagat aaagcttctg gatttcacta
 541 tggagttcat gcttgtgaag gatgcaaggg tttcttccgg agaacaatca gattgaagct
 601 tatctatgac agatgtgatc ttaactgtcg gatccacaaa aaaagtagaa ataaatgtca
 661 gtactgtcgg tttcagaaat gccttgacgt ggggatgtct cataatgcc a tcaggtttgg
 721 gcggatcgca caggccgaga aggagaagct gttggcggag atctccagtg atatcgacca
 781 gctgaatcca gagtccgctg acctccgtca ggccctggca aaacatttgt atgactcata
 841 cataaagtcc tccccgtga ccaaagcaaa ggcgagggcg atcttgacag gaaagacaac
 901 agacaaatca ccattcgtaa tctatgacat gaattcctta atgatgggag aagataaaat
 961 caagttcaaa cacatcacc cctgcagga gcagagcaaa gaggtggcca tccgcattct
 1021 tcagggctgc cagtttcgct ccgtggaggc tgtgcaggag atcacagagt atgccaaaag
 1081 cattcctggt tttgtaaatc ttgacttgaa cgaccaagta actctcctca aatatggagt
 1141 ccacgagatc atttacacaa tgctggcctc cttgatgaat aaagatggg tttctcatatc
 1201 cgagggccaa ggcttcatga caaggaggt tctaaagagc ctgcgaaagc cttttggtga
 1261 ctttatggag cccaagtttg agtttgctgt gaagttcaat gcactggaat tagatgacag
 1321 cgacttgga atatttattg ctgtcattat tctcagtga gaccgccag gtttgctgaa
 1381 tgtgaagccc attgaagaca ttcaagacaa cctgctacaa gccctggagc tccagctgaa
 1441 gctgaaccac cctgagtcct cacagctgtt tgccaagctg ctccagaaaa tgacagacct
 1501 cagacagatt gtcacggaac acgtgcagct actgcagggt atcaagaaga cggagacaga
 1561 catgagtctt caccgctcc tgcaggagat ctacaaggac ttgtactagc agagagtcct
 1621 gagccactgc caacatttcc cttcttccag ttgcactatt ctgagggaaa atctgaccat
 1681 aagaaattta ctgtgaaaaa gcgttttaaa aagaaaaggg tttagaatat gatctatatt
 1741 atgcatattg tttataaaga cacatttaca atttactttt aatattaata attaccatat
 1801 tatgaaattg c

FIG.9B

9/11

1 MEQPQEEAPEVREEEEEKEEVAEAEGAPELNGGPQHALPSSSYTDLSRSSSPPSLLDQLQM
61 GCPGASCGSLNMECRVCGDKASGFHYGVHACEGCKGFFRRTIRMKLEYEKCERSCKIQKK
121 NRNKCQYCRFQKCLALGMSHNAIRFGRMPEAEKRKL VAGLTANEGSQYNPQVADLKAFSK
181 HIYNAYLKNFNMTKKKARSILTGKASHTAPFVIHDIETLWQAEKGLVWKQLVNGLPPYKE
241 ISVHVFYRCQCTTVETVRELTEFAKSIPSFSSLFLNDQVTLLKYGVHEAIFAMLASIVNK
301 DGLLVANGSGFVTREFLRSLRKPFSDIIEPKFEFAVKFNALELDDSDLALFIAAIIILCGD
361 RPGLMNVPRVEAIQDTILRALEFHLQANHPDAQYLFPKLLQKMADLRQLVTEHAQMMQRI
421 KKTETETSLHPLLQEIKDMY

FIG.10A

10/11

```

1   gaattctgcg gagcctgcgg gacggcggcg ggttggcccc taggcagccg ggacagtgtt
61  gtacagtgtt ttgggcatgc acgtgatact cacacagtgg cttctgctca ccaacagatg
121 aagacagatg caccaacgag ggtctggaat ggtctggagt ggtctggaaa gcagggtcag
181 atacccttgg aaaactgaag cccgtggagc aatgatctct acaggactgc ttcaaggctg
241 atgggaacca ccctgtagag gtccatctgc gttcagaccc agacgatgcc agagctatga
301 ctgggcctgc aggtgtggcg ccgaggggag atcagccatg gagcagccac aggaggaagc
361 ccctgaggtc cgggaagagg aggagaaaga ggaagtggca gaggcagaag gagccccaga
421 gctcaatggg ggaccacagc atgcacttcc ttccagcagc tacacagacc tctcccggag
481 ctctctgcca ccctcactgc tggaccaact gcagatgggc tgtgacgggg cctcatgcgg
541 cagcctcaac atggagtgcc ggggtgtgcg ggacaaggca tcgggcttcc actacggtgt
601 tcatgcatgt gaggggtgca agggcttctt ccgtcgtacg atccgcatga agctggagta
661 cgagaagtgt gagcgcagct gcaagattca gaagaagaac cgcaacaagt gccagtactg
721 ccgcttccag aagtgcctgg cactgggcat gtcacacaac gctatccgtt ttggtcggat
781 gccggaggct gagaagagga agctggtggc agggctgact gcaaacgagg ggagccagta
841 caaccacag gtggccgacc tgaaggcctt ctccaagcac atctacaatg cctacctgaa
901 aaacttcaac atgacaaaaa agaaggcccg cagcatcctc accggcaaag ccagccacac
961 ggcgcctttt gtgatccacg acatcgagac attgtggcag gcagagaagg ggctggtgtg
1021 gaagcagttg gtgaatggcc tgcctcccta caaggagatc agcgtgcacg tcttctaccg
1081 ctgccagtgc accacagtgg agaccgtgcg ggagctcact gagttcgcca agagcatccc
1141 cagcttcagc agcctcttcc tcaacgacca gggtaccctt ctcaagtatg gcgtgcacga
1201 ggccatcttc gccatgctgg cctctatcgt caacaaggac gggctgctgg tagccaacgg
1261 cagtggcttt gtcaccctg agttcctgcg cagcctccgc aaacccttca gtgatatcat
1321 tgagcctaag tttgaatttg ctgtcaagtt caacgccctg gaacttgatg acagtgaact
1381 ggccctattc attgcggcc a tcattctgtg tggagaccgg ccaggcctca tgaacgttcc
1441 acgggtggag gctatccagg acaccatcct gcgtgccctc gaattccacc tgcaggccaa
1501 ccaccctgat gccagtacc tcttcccaa gctgctgcag aagatggctg acctgcggca
1561 actggtcacc gagcacgcc agatgatgca gcggatcaag aagaccgaaa ccpagacctc
1621 gctgcacct ctgctccagg agatctacaa ggacatgtac taacggcggc acccaggcct
1681 ccctgcagac tccaatgggg ccagcactgg aggggcccac ccacatgact tttccattga
1741 ccagctctct tctgtcttt gtgtctctcc tctttctcag ttctctttc ttttctaatt
1801 cctgttgctc tgtttcttcc tttctgtagg tttctctctt ccctctccc ttctcccttg
1861 ccctcccttt ctctctccta tccccacgtc tgtcctcctt tcttattctg tgagatgttt
1921 tgtattattt caccagcagc atagaacagg acctctgctt ttgcacacct tttccccagg
1981 agcagaagag agtgggcctg ccctctgccc catcattgca cctgcaggct taggtcctca
2041 cttctgtctc ctgtcttcag agcaaaagac ttgagccatc caaagaaaca ctaagctctc
2101 tgggcctggg ttccagggaa ggctaagcat ggcctggact gactgcagcc ccctatagtc
2161 atgggggtccc tgctgcaaa gacagtggca gaccccgcca gtagagccga gatgcctccc
2221 caagactgtc attgcccctc cgatcgtgag gccaccact gacccaatga tctctccag
2281 cagcacacct cagccccact gacaccagc gtccttccat cttcacactg gtttgccagg
2341 ccaatgttgc tgatggcccc tccagcacac acacataagc actgaaatca ctttacctgc
2401 aggcaccatg cacctccctt ccctccctga ggcaggtag aaccagaga gaggggcctg

```

FIG. 10B

11/11

2461 caggtgagca ggcagggctg ggccaggtct ccggggaggc aggggtcctg caggtcctgg
2521 tgggtcagcc cagcacctcg cccagtggga gcttcccggg ataaactgag cctgttcatt
2581 ctgatgtcca tttgtcccaa tagctctact gccctcccct tcccctttac tcagcccagc
2641 tggccaccta gaagtctccc tgcacagcct ctagtgtccg gggaccttgt gggaccagtc
2701 ccacaccgct ggtccctgcc ctcccctgct cccaggttga ggtgcgctca cctcagagca
2761 gggccaaagc acagctgggc atgccatgtc tgagcggcgc agagccctcc aggcctgcag
2821 gggcaagggg ctggctggag tctcagagca cagaggtagg agaactgggg ttcaagccca
2881 ggcttcctgg gtcctgcctg gtcctccctc ccaaggagcc attctatgtg actctgggtg
2941 gaagtgccca gcccctgcct gacggnnnnn nngatcactc tctgctggca ggattcttcc
3001 cgctccccac ctaccagct gatgggggtt ggggtgcttc tttcagccaa ggctatgaag
3061 ggacagctgc tgggaccac ctccccctt ccccgccac atgccgcgtc cctgccccca
3121 cccgggtctg gtgctgagga tacagctctt ctcagtgtct gaacaatctc caaaattgaa
3181 atgtatattt ttgctaggag cccagcttc ctgtgttttt aatataaata gtgtacacag
3241 actgacgaaa ctttaaataa atgggaatta aatattttaa aaaaaagcg gccgcgaatt
3301 c

FIG.10C

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Merck & Co., Inc.
- (ii) TITLE OF INVENTION: ASSAYS FOR NUCLEAR RECEPTOR
AGONISTS AND ANTAGONISTS USING FLUORESCENCE RESONANCE
ENERGY TRANSFER
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Merck & Co., Inc.
 - (B) STREET: P.O. Box 2000, 126 E. Lincoln Ave.
 - (C) CITY: Rahway
 - (D) STATE: NJ
 - (E) COUNTRY: USA
 - (F) ZIP: 07065-0900
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: Windows
 - (D) SOFTWARE: FastSEQ for Windows Version 2.0b
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Coppola, Joseph A
 - (B) REGISTRATION NUMBER: 38,413
 - (C) REFERENCE/DOCKET NUMBER: 20017PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 732-594-6734
 - (B) TELEFAX: 732-594-4720
 - (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 405 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

Met Ala Glu Asn Leu Leu Asp Gly Pro Pro Asn Pro Lys Arg Ala Lys
 1      5      10      15
Leu Ser Ser Pro Gly Phe Ser Ala Asn Asp Ser Thr Asp Phe Gly Ser
 20      25      30
Leu Phe Asp Leu Glu Asn Asp Leu Pro Asp Glu Leu Ile Pro Asn Gly
 35      40      45
Gly Glu Leu Gly Leu Leu Asn Ser Gly Asn Leu Val Pro Asp Ala Ala
 50      55      60
Ser Lys His Lys Gln Leu Ser Glu Leu Leu Arg Gly Gly Ser Gly Ser
 65      70      75      80
Ser Ile Asn Pro Gly Ile Gly Asn Val Ser Ala Ser Ser Pro Val Gln
 85      90      95
Gln Gly Leu Gly Gly Gln Ala Gln Gly Gln Pro Asn Ser Ala Asn Met
100      105      110
Ala Ser Leu Ser Ala Met Gly Lys Ser Pro Leu Ser Gln Gly Asp Ser
115      120      125
Ser Ala Pro Ser Leu Pro Lys Gln Ala Ala Ser Thr Ser Gly Pro Thr
130      135      140
Pro Ala Ala Ser Gln Ala Leu Asn Pro Gln Ala Gln Lys Gln Val Gly
145      150      155      160
Leu Ala Thr Ser Ser Pro Ala Thr Ser Gln Thr Gly Pro Gly Ile Cys
165      170      175
Met Asn Ala Asn Phe Asn Gln Thr His Pro Gly Leu Leu Asn Ser Asn
180      185      190
Ser Gly His Ser Leu Ile Asn Gln Ala Ser Gln Gly Gln Ala Gln Val
195      200      205
Met Asn Gly Ser Leu Gly Ala Ala Gly Arg Gly Arg Gly Ala Gly Met
210      215      220
Pro Tyr Pro Thr Pro Ala Met Gln Gly Ala Ser Ser Ser Val Leu Ala
225      230      235      240
Glu Thr Leu Thr Gln Val Ser Pro Gln Met Thr Gly His Ala Gly Leu
245      250      255
Asn Thr Ala Gln Ala Gly Gly Met Ala Lys Met Gly Ile Thr Gly Asn
260      265      270
Thr Ser Pro Phe Gly Gln Pro Phe Ser Gln Ala Gly Gly Gln Pro Met
275      280      285
Gly Ala Thr Gly Val Asn Pro Gln Leu Ala Ser Lys Gln Ser Met Val
290      295      300
Asn Ser Leu Pro Thr Phe Pro Thr Asp Ile Lys Asn Thr Ser Val Thr
305      310      315      320
Asn Val Pro Asn Met Ser Gln Met Gln Thr Ser Val Gly Ile Val Pro
325      330      335
Thr Gln Ala Ile Ala Thr Gly Pro Thr Ala Asp Pro Glu Lys Arg Lys
340      345      350
Leu Ile Gln Gln Gln Leu Val Leu Leu His Ala His Lys Cys Gln
355      360      365
Arg Arg Glu Gln Ala Asn Gly Glu Val Arg Ala Cys Ser Leu Pro His
370      375      380
Cys Arg Thr Met Lys Asn Val Leu Asn His Met Thr His Cys Gln Ala
385      390      395      400
Gly Lys Ala Cys Gln
405

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1290 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

CGAGCCCCGA CCCCCGTCCG GGCCCTCGCC GGCCGCGCCG CCCGTGCCCCG GGGCTGTTTTT    60
CCCCGAGCAGG TGAAAATGGC TGAGAACTTG CTGGACGGAC CGCCCAACCC CAAAAGAGCC    120
AAACTCAGCT CGCCCGGTTT CTCGGCGAAT GACAGCACAG ATTTTGGATC ATGTGTTGAC    180
TTGGAAAATG ATCTTCCAGA TGAGCTGATA CCAATGGAG GAGAATTAGG CCTTTTAAAC    240
AGTGGGAACC TTGTTCCAGA TGCTGCTTCC AAACATAAAC AACTGTCGGA GCTTCTACGA    300
GGAGGCAGCG GCTCTAGTAT CAACCCAGGA ATAGGAAATG TGAGCGCCAG CAGCCCCGTG    360
CAGCAGGGCC TGGGTGGCCA GGCTCAAGGG CAGCCGAACA GTGCTAACAT GGCCAGCCTC    420
AGTGCCATGG GCAAGAGCCC TCTGAGCCAG GGAGATTCTT CAGCCCCCAG CCTGCCTAAA    480
CAGGCAGCCA GCACCTCTGG GCCCACCCCC GCTGCCTCCC AAGCACTGAA TCCGCAAGCA    540
CAAAAGCAAG TGGGGCTGGC GACTAGCAGC CCTGCCACGT CACAGACTGG ACCTGGTATC    600
TGCAAGAATG CTAACTTTAA CCAGACCCAC CCAGGCCTCC TCAATAGTAA CTCTGGCCAT    660
AGCTTAATTA ATCAGGCTTC ACAAGGGCAG GCGCAAGTCA TGAATGGATC TCTTGGGGCT    720
GCTGGCAGAG GAAGGGGAGC TGAATGCCG TACCCTACTC CAGCCATGCA GGGCGCCTCG    780
AGCAGCGTGC TGGCTGAGAC CCTAACGCAG GTTTCCCCGC AAATGACTGG TCACGCGGGA    840
CTGAACACCG CACAGGCAGG AGGCATGGCC AAGATGGGAA TAACTGGGAA CACAAGTCCA    900
TTTGGACAGC CCTTTAGTCA AGCTGGAGGG CAGCCAATGG GAGCCACTGG AGTGAACCCC    960
CAGTTAGCCA GCAAACAGAG CATGGTCAAC AGTTTGCCCA CCTTCCCTAC AGATATCAAG   1020
AATACTTCAG TCACCAACGT GCCAAATATG TCTCAGATGC AAACATCAGT GGAATTTGTA   1080
CCCACACAAG CAATTGCAAC AGGCCCCACT GCAGATCCTG AAAAAACGAA ACTGATACAG   1140
CAGCAGCTGG TTCTACTGCT TCATGCTCAT AAGTGTGAGA GACGAGAGCA AGCAAACGGA   1200
GAGGTTCTGGG CCTGCTCGCT CCCGCATTGT CGAACCATGA AAAACGTTTTT GAATCACATG   1260
ACGCATTGTC AGGCTGGGAA AGCCTGCCAA

```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 468 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Val Asp Thr Glu Ser Pro Leu Cys Pro Leu Ser Pro Leu Glu Ala
 1           5           10           15
Gly Asp Leu Glu Ser Pro Leu Ser Glu Glu Phe Leu Gln Glu Met Gly
          20          25          30
Asn Ile Gln Glu Ile Ser Gln Ser Ile Gly Glu Asp Ser Ser Gly Ser
          35          40          45
Phe Gly Phe Thr Glu Tyr Gln Tyr Leu Gly Ser Cys Pro Gly Ser Asp
          50          55          60

```

Gly	Ser	Val	Ile	Thr	Asp	Thr	Leu	Ser	Pro	Ala	Ser	Ser	Pro	Ser	Ser	
65					70					75					80	
Val	Thr	Tyr	Pro	Val	Val	Pro	Gly	Ser	Val	Asp	Glu	Ser	Pro	Ser	Gly	
				85					90					95		
Ala	Leu	Asn	Ile	Glu	Cys	Arg	Ile	Cys	Gly	Asp	Lys	Ala	Ser	Gly	Tyr	
			100					105					110			
His	Tyr	Gly	Val	His	Ala	Cys	Glu	Gly	Cys	Lys	Gly	Phe	Phe	Arg	Arg	
		115					120					125				
Thr	Ile	Arg	Leu	Lys	Leu	Val	Tyr	Asp	Lys	Cys	Asp	Arg	Ser	Cys	Lys	
		130				135					140					
Ile	Gln	Lys	Lys	Asn	Arg	Asn	Lys	Cys	Gln	Tyr	Cys	Arg	Phe	His	Lys	
145				150					155						160	
Cys	Leu	Ser	Val	Gly	Met	Ser	His	Asn	Ala	Ile	Arg	Phe	Gly	Arg	Met	
			165					170						175		
Pro	Arg	Ser	Glu	Lys	Ala	Lys	Leu	Lys	Ala	Glu	Ile	Leu	Thr	Cys	Glu	
			180					185					190			
His	Asp	Ile	Glu	Asp	Ser	Glu	Thr	Ala	Asp	Leu	Lys	Ser	Leu	Ala	Lys	
		195				200						205				
Arg	Ile	Tyr	Glu	Ala	Tyr	Leu	Lys	Asn	Phe	Asn	Met	Asn	Lys	Val	Lys	
	210					215					220					
Ala	Arg	Val	Ile	Leu	Ser	Gly	Lys	Ala	Ser	Asn	Asn	Pro	Pro	Phe	Val	
225				230						235					240	
Ile	His	Asp	Met	Glu	Thr	Leu	Cys	Met	Ala	Glu	Lys	Thr	Leu	Val	Ala	
			245						250					255		
Lys	Leu	Val	Ala	Asn	Gly	Ile	Gln	Asn	Lys	Glu	Val	Glu	Val	Arg	Ile	
		260					265					270				
Phe	His	Cys	Cys	Gln	Cys	Thr	Ser	Val	Glu	Thr	Val	Thr	Glu	Leu	Thr	
		275				280						285				
Glu	Phe	Ala	Lys	Ala	Ile	Pro	Ala	Phe	Ala	Asn	Leu	Asp	Leu	Asn	Asp	
	290					295				300						
Gln	Val	Thr	Leu	Leu	Lys	Tyr	Gly	Val	Tyr	Glu	Ala	Ile	Phe	Ala	Met	
305				310						315					320	
Leu	Ser	Ser	Val	Met	Asn	Lys	Asp	Gly	Met	Leu	Val	Ala	Tyr	Gly	Asn	
			325					330						335		
Gly	Phe	Ile	Thr	Arg	Glu	Phe	Leu	Lys	Ser	Leu	Arg	Lys	Pro	Phe	Cys	
		340					345					350				
Asp	Ile	Met	Glu	Pro	Lys	Phe	Asp	Phe	Ala	Met	Lys	Phe	Asn	Ala	Leu	
		355				360						365				
Glu	Leu	Asp	Asp	Ser	Asp	Ile	Ser	Leu	Phe	Val	Ala	Ala	Ile	Ile	Cys	
	370					375					380					
Cys	Gly	Asp	Arg	Pro	Gly	Leu	Leu	Asn	Val	Gly	His	Ile	Glu	Lys	Met	
385				390					395						400	
Gln	Glu	Gly	Ile	Val	His	Val	Leu	Arg	Leu	His	Leu	Gln	Ser	Asn	His	
			405					410						415		
Pro	Asp	Asp	Ile	Phe	Leu	Phe	Pro	Lys	Leu	Leu	Gln	Lys	Met	Ala	Asp	
			420					425					430			
Leu	Arg	Gln	Leu	Val	Thr	Glu	His	Ala	Gln	Leu	Val	Gln	Ile	Ile	Lys	
		435				440						445				
Lys	Thr	Glu	Ser	Asp	Ala	Ala	Leu	His	Pro	Leu	Leu	Gln	Glu	Ile	Tyr	
	450					455					460					
Arg	Asp	Met	Tyr													
465																

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1854 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

GGCCCAGGCT GAAGCTCAGG GCCCTGTCTG CTCTGTGGAC TCAACAGTTT GTGGCAAGAC      60
AAGCTCAGAA CTGAGAAGCT GTCACCACAG TTCTGGAGGC TGGGAAGTTC AAGATCAAAG      120
TGCCAGCAGA TTCAGTGTC A GTGAGGACG TGCTTCCTGC TTCATAGATA AGAGTAGCTT      180
GGAGCTCGGC GGCACAACCA GCACCATCTG GTCGCGATGG TGGACACGGA AAGCCCCTC      240
TGCCCCCTCT CCCCCTCGA GGCCGGCGAT CTAGAGAGCC CGTTATCTGA AGAGTTCCTG      300
CAAGAAATGG GAAACATCCA AGAGATTTTC CAATCCATCG GCGAGGATAG TTCTGGAAGC      360
TTTGGCTTTA CGGAATACCA GTATTTAGGA AGCTGTCTCT GCTCAGATGG CTCGGTCATC      420
ACGGACACGC TTTCACCAGC TTCGAGCCCC TCCTCGGTGA CTTATCCTGT GGTCCCCGGC      480
AGCGTGGACG AGTCTCCAG TGGAGCATTG AACATCGAAT GTAGAATCTG CGGGGACAAG      540
GCCTCAGGCT ATCATTACGG AGTCCACGCG TGTGAAGGCT GCAAGGGCTT CTTTCGGCGA      600
ACGATTCGAC TCAAGCTGGT GTATGACAAG TGCGACCGCA GCTGCAAGAT CCAGAAAAAG      660
AACAGAAACA AATGCCAGTA TTGTCGATTT CACAAGTGCC TTTCTGTCGG GATGTCACAC      720
AACGCGATTC GTTTTGGACG AATGCCAAGA TCTGAGAAAG CAAAAGTGAA AGCAGAAATT      780
CTTACCTGTG AACATGACAT AGAAGATTCT GAAACTGCAG ATCTCAAATC TCTGGCCAAG      840
AGAATCTACG AGGCCTACTT GAAGAACTTC AACATGAACA AGGTCAAAGC CCGGGTCATC      900
CTCTCAGGAA AGGCCAGTAA CAATCCACCT TTTGTCATAC ATGATATGGA GACACTGTGT      960
ATGGCTGAGA AGACGCTGGT GGCCAAGCTG GTGGCCAATG GCATCCAGAA CAAGGAGGTG     1020
GAGGTCCGCA TCTTTCACTG CTGCCAGTGC ACGTCAGTGG AGACCGTCAC GGAGCTCACG     1080
GAATTCGCCA AGGCCATCCC AGCGTTCGCA AACTTGGACC TGAACGATCA AGTGACATTG     1140
CTAAAATACG GAGTTTATGA GGCCATATTC GCCATGCTGT CTTCTGTGAT GAACAAAGAC     1200
GGGATGCTGG TAGCGTATGG AAATGGGTTT ATAACTCGTG AATTCCTAAA AAGCCTAAGG     1260
AAACCGTTCT GTGATATCAT GGAACCCAAG TTTGATTTTG CCATGAAGTT CAATGCACTG     1320
GAACTGGATG ACAGTGATAT CTCCCTTTTT GTGGCTGCTA TCATTTGCTG TGGAGATCGT     1380
CCTGGCCTTC TAAACGTAGG ACACATTGAA AAAATGCAGG AGGGTATTGT ACATGTGCTC     1440
AGACTCCACC TGCAGAGCAA CCACCCGGAC GATATCTTTC TCTTCCCAA ACTTCTTCAA     1500
AAAATGGCAG ACCTCCGGCA GCTGGTGACG GAGCATGCGC AGCTGGTGCA GATCATCAAG     1560
AAGACGGAGT CGGATGCTGC GCTGCACCCG CTACTGCAGG AGATCTACAG GGACATGTAC     1620
TGAGTTCCTT CAGATCAGCC ACACCTTTTC CAGGAGTTCT GAAGCTGACA GCACTACAAA     1680
GGAGACGGGG GAGCAGCAGC ATTTTGCACA AATATCCACC ACTTTAACCT TAGAGCTTGG     1740
ACAGTCTGAG CTGTAGGTAA CCGGCATATT ATTCCATATC TTTGTTTTAA CCAGTACTTC     1800
TAAGAGCATA GAACTCAAAT GCTGGGGGAG GTGGCTAATC TCAGGACTGG GAAG           1854
  
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 478 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Met Thr Met Val Asp Thr Glu Ile Ala Phe Trp Pro Thr Asn Phe Gly
 1             5             10             15
  
```

Ile	Ser	Ser	Val	Asp	Leu	Ser	Val	Met	Glu	Asp	His	Ser	His	Ser	Phe
			20					25					30		
Asp	Ile	Lys	Pro	Phe	Thr	Thr	Val	Asp	Phe	Ser	Ser	Ile	Ser	Thr	Pro
		35					40					45			
His	Tyr	Glu	Asp	Ile	Pro	Phe	Thr	Arg	Thr	Asp	Pro	Val	Val	Ala	Asp
		50				55					60				
Tyr	Lys	Tyr	Asp	Leu	Lys	Leu	Gln	Glu	Tyr	Gln	Ser	Ala	Ile	Lys	Val
65				70						75					80
Glu	Pro	Ala	Ser	Pro	Tyr	Tyr	Ser	Glu	Lys	Thr	Gln	Leu	Tyr	Asn	
			85					90					95		
Lys	Pro	His	Glu	Glu	Pro	Ser	Asn	Ser	Leu	Met	Ala	Ile	Glu	Cys	Arg
			100					105					110		
Val	Cys	Gly	Asp	Lys	Ala	Ser	Gly	Phe	His	Tyr	Gly	Val	His	Ala	Cys
		115					120					125			
Glu	Gly	Cys	Lys	Gly	Phe	Phe	Arg	Arg	Thr	Ile	Arg	Leu	Lys	Leu	Ile
		130				135					140				
Tyr	Asp	Arg	Cys	Asp	Leu	Asn	Cys	Arg	Ile	His	Lys	Lys	Ser	Arg	Asn
145					150					155					160
Lys	Cys	Gln	Tyr	Cys	Arg	Phe	Gln	Lys	Cys	Leu	Ala	Val	Gly	Met	Ser
			165					170						175	
His	Asn	Ala	Ile	Arg	Phe	Gly	Arg	Ile	Ala	Gln	Ala	Glu	Lys	Glu	Lys
			180					185					190		
Leu	Leu	Ala	Glu	Ile	Ser	Ser	Asp	Ile	Asp	Gln	Leu	Asn	Pro	Glu	Ser
		195					200					205			
Ala	Asp	Leu	Arg	Gln	Ala	Leu	Ala	Lys	His	Leu	Tyr	Asp	Ser	Tyr	Ile
		210				215					220				
Lys	Ser	Phe	Pro	Leu	Thr	Lys	Ala	Lys	Ala	Arg	Ala	Ile	Leu	Thr	Gly
225					230					235					240
Lys	Thr	Thr	Asp	Lys	Ser	Pro	Phe	Val	Ile	Tyr	Asp	Met	Asn	Ser	Leu
			245					250					255		
Met	Met	Gly	Glu	Asp	Lys	Ile	Lys	Phe	Lys	His	Ile	Thr	Pro	Leu	Gln
		260						265					270		
Glu	Gln	Ser	Lys	Glu	Val	Ala	Ile	Arg	Ile	Phe	Gln	Gly	Cys	Gln	Phe
		275					280					285			
Arg	Ser	Val	Glu	Ala	Val	Gln	Glu	Ile	Thr	Glu	Tyr	Ala	Lys	Ser	Ile
		290				295					300				
Pro	Gly	Phe	Val	Asn	Leu	Asp	Leu	Asn	Asp	Gln	Val	Thr	Leu	Leu	Lys
305					310					315					320
Tyr	Gly	Val	His	Glu	Ile	Ile	Tyr	Thr	Met	Leu	Ala	Ser	Leu	Met	Asn
			325					330						335	
Lys	Asp	Gly	Val	Leu	Ile	Ser	Glu	Gly	Gln	Gly	Phe	Met	Thr	Arg	Glu
			340					345					350		
Phe	Leu	Lys	Ser	Leu	Arg	Lys	Pro	Phe	Gly	Asp	Phe	Met	Glu	Pro	Lys
		355					360					365			
Phe	Glu	Phe	Ala	Val	Lys	Phe	Asn	Ala	Leu	Glu	Leu	Asp	Asp	Ser	Asp
		370				375					380				
Leu	Ala	Ile	Phe	Ile	Ala	Val	Ile	Ile	Leu	Ser	Gly	Asp	Arg	Pro	Gly
385					390					395					400
Leu	Leu	Asn	Val	Lys	Pro	Ile	Glu	Asp	Ile	Gln	Asp	Asn	Leu	Leu	Gln
			405					410						415	
Ala	Leu	Glu	Leu	Gln	Leu	Lys	Leu	Asn	His	Pro	Glu	Ser	Ser	Gln	Leu
			420					425					430		
Phe	Ala	Lys	Leu	Leu	Gln	Lys	Met	Thr	Asp	Leu	Arg	Gln	Ile	Val	Thr
		435					440					445			
Glu	His	Val	Gln	Leu	Leu	Gln	Val	Ile	Lys	Lys	Thr	Glu	Thr	Asp	Met
		450				455						460			

Ser Leu His Pro Leu Leu Gln Glu Ile Tyr Lys Asp Leu Tyr
465 470 475

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1811 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCGACCTTAC	CCCAGGCGGC	CTTGACGTTG	GTCTTGTCGG	CAGGAGACAG	CACCATGGTG	60
GGTTCTCTCT	GAGTCTGGGA	ATTCCCAGAGC	CCGAGCCGCA	GCCGCCGCCT	GGGGGGCTTG	120
GGTCGGCCTC	GAGGACACCG	GAGAGGGGCG	CCACGCCGCC	GTGGCCGCAG	AAATGACCAT	180
GGTTGACACA	GAGATCGCAT	TCTGGCCCAC	CAACTTTGGG	ATCAGCTCCG	TGGATCTCTC	240
CGTAATGGAA	GACCACTCCC	ACTCCTTTGA	TATCAAGCCC	TTCCTACTG	TTGACTTCTC	300
CAGCATTTCT	ACTCCACATT	ACGAAGACAT	TCCATTACACA	AGAACAGATC	CAGTGGTTGC	360
AGATTACAAG	TATGACCTGA	AACTTCAAGA	GTACCAAAGT	GCAATCAAAG	TGGAGCCTGC	420
ATCTCCACCT	TATTATTCTG	AGAAGACTCA	GCTCTACAAT	AAGCCTCATG	AAGAGCCTTC	480
CAACTCCCTC	ATGGCAATTG	AATGTCGTGT	CTGTGGAGAT	AAAGCTTCTG	GATTTCACTA	540
TGGAGTTTCAT	GCTTGTGAAG	GATGCAAGGG	TTTCTTCCGG	AGAACAATCA	GATTGAAGCT	600
TATCTATGAC	AGATGTGATC	TTAACTGTCTG	GATCCACAAA	AAAAGTAGAA	ATAAATGTCA	660
GTACTGTCTGG	TTTCAGAAAT	GCCTTGCACT	GGGGATGTCT	CATAATGCCA	TCAGGTTTGG	720
GCGGATCGCA	CAGGCCGAGA	AGGAGAAGCT	GTTGGCGGAG	ATCTCCAGTG	ATATCGACCA	780
GCTGAATCCA	GAGTCCGCTG	ACCTCCGTCA	GGCCCTGGCA	AAACATTTGT	ATGACTCATA	840
CATAAAGTCC	TTCCCGCTGA	CCAAAGCAAA	GGCGAGGGCG	ATCTTGACAG	GAAAGACAAC	900
AGACAAATCA	CCATTCGTTA	TCTATGACAT	GAATTCCTTA	ATGATGGGAG	AAGATAAAAT	960
CAAGTTCAAA	CACATCACCC	CCCTGCAGGA	GCAGAGCAAA	GAGGTGGCCA	TCCGCATCTT	1020
TCAGGGCTGC	CAGTTTCGCT	CCGTGGAGGC	TGTGCAGGAG	ATCACAGAGT	ATGCCAAAAG	1080
CATTCCCTGGT	TTTGTAATC	TTGACTTGAA	CGACCAAGTA	ACTCTCCTCA	AATATGGAGT	1140
CCACGAGATC	ATTTACACAA	TGCTGGCCTC	CTTGATGAAT	AAAGATGGGG	TTCTCATATC	1200
CGAGGGCCAA	GGCTTCATGA	CAAGGGAGTT	TCTAAAGAGC	CTGCCAAAGC	CTTTTGGTGA	1260
CTTTATGGAG	CCCAAGTTTG	AGTTTGCTGT	GAAGTTCAAT	GCACTGGAAT	TAGATGACAG	1320
CGACTTGGCA	ATATTTATTG	CTGTCATTAT	TCTCAGTGGA	GACCGCCCAG	GTTTGCTGAA	1380
TGTGAAGCCC	ATTGAAGACA	TTCAAGACAA	CCTGCTACAA	GCCCTGGAGC	TCCAGCTGAA	1440
GCTGAACCAC	CCTGAGTCCT	CACAGCTGTT	TGCCAAGCTG	CTCCAGAAAA	TGACAGACCT	1500
CAGACAGATT	GTCACGGAAC	ACGTGCAGCT	ACTGCAGGTG	ATCAAGAAGA	CGGAGACAGA	1560
CATGAGTCTT	CACCCGCTCC	TGCAGGAGAT	CTACAAGGAC	TTGTACTAGC	AGAGAGTCCT	1620
GAGCCACTGC	CAACATTTCC	CTTCTTCCAG	TTGCACTATT	CTGAGGGAAA	ATCTGACCAT	1680
AAGAAATTTA	CTGTGAAAAA	GCGTTTTTAA	AAGAAAAGGG	TTTAGAATAT	GATCTATTTT	1740
ATGCATATTG	TTTATAAAGA	CACATTTACA	ATTTACTTTT	AATATTAAAA	ATTACCATAT	1800
TATGAAATTG	C					1811

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 441 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met	Glu	Gln	Pro	Gln	Glu	Glu	Ala	Pro	Glu	Val	Arg	Glu	Glu	Glu	Glu
1				5					10					15	
Lys	Glu	Glu	Val	Ala	Glu	Ala	Glu	Gly	Ala	Pro	Glu	Leu	Asn	Gly	Gly
			20					25					30		
Pro	Gln	His	Ala	Leu	Pro	Ser	Ser	Tyr	Thr	Asp	Leu	Ser	Arg	Ser	
		35					40				45				
Ser	Ser	Pro	Pro	Ser	Leu	Leu	Asp	Gln	Leu	Gln	Met	Gly	Cys	Asp	Gly
	50					55					60				
Ala	Ser	Cys	Gly	Ser	Leu	Asn	Met	Glu	Cys	Arg	Val	Cys	Gly	Asp	Lys
65					70					75					80
Ala	Ser	Gly	Phe	His	Tyr	Gly	Val	His	Ala	Cys	Glu	Gly	Cys	Lys	Gly
				85					90					95	
Phe	Phe	Arg	Arg	Thr	Ile	Arg	Met	Lys	Leu	Glu	Tyr	Glu	Lys	Cys	Glu
			100					105					110		
Arg	Ser	Cys	Lys	Ile	Gln	Lys	Lys	Asn	Arg	Asn	Lys	Cys	Gln	Tyr	Cys
		115					120					125			
Arg	Phe	Gln	Lys	Cys	Leu	Ala	Leu	Gly	Met	Ser	His	Asn	Ala	Ile	Arg
	130					135					140				
Phe	Gly	Arg	Met	Pro	Glu	Ala	Glu	Lys	Arg	Lys	Leu	Val	Ala	Gly	Leu
145					150					155					160
Thr	Ala	Asn	Glu	Gly	Ser	Gln	Tyr	Asn	Pro	Gln	Val	Ala	Asp	Leu	Lys
				165				170						175	
Ala	Phe	Ser	Lys	His	Ile	Tyr	Asn	Ala	Tyr	Leu	Lys	Asn	Phe	Asn	Met
			180				185						190		
Thr	Lys	Lys	Lys	Ala	Arg	Ser	Ile	Leu	Thr	Gly	Lys	Ala	Ser	His	Thr
		195					200					205			
Ala	Pro	Phe	Val	Ile	His	Asp	Ile	Glu	Thr	Leu	Trp	Gln	Ala	Glu	Lys
	210					215					220				
Gly	Leu	Val	Trp	Lys	Gln	Leu	Val	Asn	Gly	Leu	Pro	Pro	Tyr	Lys	Glu
225					230					235					240
Ile	Ser	Val	His	Val	Phe	Tyr	Arg	Cys	Gln	Cys	Thr	Thr	Val	Glu	Thr
				245					250					255	
Val	Arg	Glu	Leu	Thr	Glu	Phe	Ala	Lys	Ser	Ile	Pro	Ser	Phe	Ser	Ser
			260					265					270		
Leu	Phe	Leu	Asn	Asp	Gln	Val	Thr	Leu	Leu	Lys	Tyr	Gly	Val	His	Glu
	275						280					285			
Ala	Ile	Phe	Ala	Met	Leu	Ala	Ser	Ile	Val	Asn	Lys	Asp	Gly	Leu	Leu
	290					295					300				
Val	Ala	Asn	Gly	Ser	Gly	Phe	Val	Thr	Arg	Glu	Phe	Leu	Arg	Ser	Leu
305					310					315					320
Arg	Lys	Pro	Phe	Ser	Asp	Ile	Ile	Glu	Pro	Lys	Phe	Glu	Phe	Ala	Val
				325					330					335	
Lys	Phe	Asn	Ala	Leu	Glu	Leu	Asp	Asp	Ser	Asp	Leu	Ala	Leu	Phe	Ile
			340					345					350		
Ala	Ala	Ile	Ile	Leu	Cys	Gly	Asp	Arg	Pro	Gly	Leu	Met	Asn	Val	Pro
		355					360					365			
Arg	Val	Glu	Ala	Ile	Gln	Asp	Thr	Ile	Leu	Arg	Ala	Leu	Glu	Phe	His
	370					375					380				
Leu	Gln	Ala	Asn	His	Pro	Asp	Ala	Gln	Tyr	Leu	Phe	Pro	Lys	Leu	Leu
385					390					395					400
Gln	Lys	Met	Ala	Asp	Leu	Arg	Gln	Leu	Val	Thr	Glu	His	Ala	Gln	Met
				405					410					415	

Met Gln Arg Ile Lys Lys Thr Glu Thr Glu Thr Ser Leu His Pro Leu
 420 425 430
 Leu Gln Glu Ile Tyr Lys Asp Met Tyr
 435 440

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3301 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAATTCTGCG	GAGCCTGCGG	GACGGCGGCG	GGTTGGCCCC	TAGGCAGCCG	GGACAGTGT	60
GTACAGTGT	TTGGGCATGC	ACGTGATACT	CACACAGTGG	CTTCTGCTCA	CCAACAGATG	120
AAGACAGATG	CACCAACGAG	GGTCTGGAAT	GGTCTGGAGT	GGTCTGGAAA	GCAGGGTCAG	180
ATACCCCTGG	AAACTGAAG	CCCGTGGAGC	AATGATCTCT	ACAGGACTGC	TTCAAGGCTG	240
ATGGGAACCA	CCCTGTAGAG	GTCCATCTGC	GTTTCAGACCC	AGACGATGCC	AGAGCTATGA	300
CTGGGCCTGC	AGGTGTGGCG	CCGAGGGGAG	ATCAGCCATG	GAGCAGCCAC	AGGAGGAAGC	360
CCCTGAGGTG	CGGGAAGAGG	AGGAGAAAGA	GGAAGTGGCA	GAGGCAGAAG	GAGCCCCAGA	420
GCTCAATGGG	GGACCACAGC	ATGCAC TTCC	TTCCAGCAGC	TACACAGACC	TCTCCCGGAG	480
CTCCTCGCCA	CCCTCACTGC	TGGACCAACT	GCAGATGGGC	TGTGACGGGG	CCTCATGCGG	540
CAGCCTCAAC	ATGGAGTGCC	GGGTGTGCGG	GGACAAGGCA	TCGGGCTTCC	ACTACGGTGT	600
TCATGCATGT	GAGGGGTGCA	AGGGCTTCTT	CCGTCGTACG	ATCCGCATGA	AGCTGGAGTA	660
CGAGAAGTGT	GAGCGCAGCT	GCAAGATTCA	GAAGAAGAAC	CGCAACAAGT	GCCAGTACTG	720
CCGCTTCCAG	AAGTGCCTGG	CAC TGGGCAT	GTCAACAAC	GCTATCCGTT	TTGGTCGGAT	780
GCCGGAGGCT	GAGAAGAGGA	AGCTGGTGCC	AGGGCTGACT	GCAAACGAGG	GGAGCCAGTA	840
CAACCCACAG	GTGGCCGACC	TGAAGGCC TT	CTCCAAGCAC	ATCTACAATG	CCTACCTGAA	900
AAACTTCAAC	ATGACCAAAA	AGAAGGCCCG	CAGCATCCTC	ACCGGCAAAG	CCAGCCACAC	960
GGCGCCCTTT	GTGATCCACG	ACATCGAGAG	ATTGTGGCAG	GCAGAGAAGG	GGCTGGTGTG	1020
GAAGCAGTTG	GTGAATGGCC	TGCC TCCCTA	CAAGGAGATC	AGCGTGCACG	TCTTCTACCG	1080
CTGCCAGTGC	ACCACAGTGG	AGACCGTGCG	GGAGCTCACT	GAGTTCGCCA	AGAGCATCCC	1140
CAGCTTCAGC	AGCCTCTTCC	TCAACGACCA	GGTTACCC TT	CTCAAGTATG	GCGTGCACGA	1200
GGCCATCTTC	GCCATGCTGG	CCTCTATCGT	CAACAAGGAC	GGGCTGCTGG	TAGCCAACGG	1260
CAGTGGCTTT	GTCACCCGTG	AGTTCC TGCG	CAGCCTCCGC	AAACCCTTCA	GTGATATCAT	1320
TGAGCCTAAG	TTTGAATTTG	CTGTCAAGTT	CAACGCCCTG	GAACTTGATG	ACAGTGACCT	1380
GGCCCTATTC	ATTGCGGCCA	TCATTCTGTG	TGGAGACCGG	CCAGGCCTCA	TGAACGTTCC	1440
ACGGGTGGAG	GCTATCCAGG	ACACCATCCT	GCGTGCCTC	GAATTCCACC	TGCAGGCCAA	1500
CCACCCTGAT	GCCAGTACC	TCTTCCCCAA	GCTGCTGCAG	AAGATGGCTG	ACCTGCGGCA	1560
ACTGGTCACC	GAGCACGCC	AGATGATGCA	GCGGATCAAG	AAGACCGAAA	CCGAGACCTC	1620
GCTGCACCCT	CTGCTCCAGG	AGATCTACAA	GGACATGTAC	TAACGGCGGC	ACCCAGGCCT	1680
CCCTGCAGAC	TCCAATGGGG	CCAGCACTGG	AGGGGCCAC	CCACATGACT	TTTCCATTGA	1740
CCAGCTCTCT	TCCTGTCTTT	GTTGTCTCCC	TCTTCTCAG	TTCTCTTTT	TTTTCTAATT	1800
CCTGTTGCTC	TGTTTCTTCC	TTTCTGTAGG	TTTCTCTCTT	CCCTTCTCCC	TTCTCCCTTG	1860
CCCTCCCTTT	CTCTCTCCTA	TCCCCACGTC	TGTCCTCCTT	TCTTATTCTG	TGAGATGTTT	1920
TGTATTATTT	CACCAGCAGC	ATAGAACAGG	ACCTCTGCTT	TTGCACACCT	TTTCCCCAGG	1980
AGCAGAAGAG	AGTGGGCCTG	CCCTCTGCCC	CATCATTGCA	CCTGCAGGCT	TAGGTCCTCA	2040
CTTCTGTCTC	CTGTCTTCAG	AGCAAAAGAC	TTGAGCCATC	CAAAGAAACA	CTAAGCTCTC	2100
TGGGCCTGGG	TTCCAGGGAA	GGCTAAGCAT	GGCCTGGACT	GACTGCAGCC	CCCTATAGTC	2160
ATGGGGTCCC	TGCTGCAAAG	GACAGTGGCA	GACCCCGGCA	GTAAGAGCCG	GATGCCTCCC	2220
CAAGACTGTC	ATTGCCCCCTC	CGATCGTGAG	GCCACCCACT	GACCCAATGA	TCCTCTCCAG	2280
CAGCACACCT	CAGCCCCACT	GACACCCAGT	GTCTTCCAT	CTTCACACTG	GTTTGCCAGG	2340

CCAATGTTGC	TGATGGCCCC	TCCAGCACAC	ACACATAAGC	ACTGAAATCA	CTTTACCTGC	2400
AGGCACCATG	CACCTCCCTT	CCCTCCCTGA	GGCAGGTGAG	AACCCAGAGA	GAGGGGCCTG	2460
CAGGTGAGCA	GGCAGGGCTG	GGCCAGGTCT	CCGGGGAGGC	AGGGGTCTTG	CAGGTCTCTG	2520
TGGGTCAGCC	CAGCACCTCG	CCCAGTGGGA	GCTTCCCGGG	ATAAACTGAG	CCTGTTTCATT	2580
CTGATGTCCA	TTTGTCCCAA	TAGCTCTACT	GCCCTCCCCT	TCCCCTTTAC	TCAGCCCAGC	2640
TGGCCACCTA	GAAGTCTCCC	TGCACAGCCT	CTAGTGTCCG	GGGACCTTGT	GGGACCAGTC	2700
CCACACCGCT	GGTCCCTGCC	CTCCCCTGCT	CCCAGGTTGA	GGTGCCTCA	CCTCAGAGCA	2760
GGGCCAAAGC	ACAGCTGGGC	ATGCCATGTC	TGAGCGGCGC	AGAGCCCTCC	AGGCCTGCAG	2820
GGGCAAGGGG	CTGGCTGGAG	TCTCAGAGCA	CAGAGGTAGG	AGAACTGGGG	TTCAAGCCCA	2880
GGCTTCCTGG	GTCTTGCTCG	GTCTTCCCTC	CCAAGGAGCC	ATTCTATGTG	ACTCTGGGTG	2940
GAAGTGCCCA	GCCCCCTGCT	GACCGMNNNN	NNGATCACTC	TCTGCTGGCA	GGATTCTTCC	3000
CGCTCCCCAC	CTACCCAGCT	GATGGGGGTT	GGGGTGCTTC	TTTCAGCCAA	GGCTATGAAG	3060
GGACAGCTGC	TGGGACCCAC	CTCCCCCCTT	CCCCGGCCAC	ATGCCGCGTC	CCTGCCCCCA	3120
CCCCGGTCTG	GTGCTGAGGA	TACAGCTCTT	CTCAGTGTCT	GAACAATCTC	CAAAATTGAA	3180
ATGTATATTT	TTGCTAGGAG	CCCCAGCTTC	CTGTGTTTTT	AATATAAATA	GTGTACACAG	3240
ACTGACGAAA	CTTTAAATAA	ATGGGAATTA	AATATTTAAA	AAAAAAAGCG	CCCGCGAATT	3300
C						3301

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACTCGGATCC	AAGCCATGGC	TGAGAACTTG	CTGGACGG	38
------------	------------	------------	----------	----

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CACAAAGCTT	AGGCCATGTT	AGCACTGTTC	GG	32
------------	------------	------------	----	----

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTCAGTCGAC TTATTGAATT CCACTAGCTG GAGATCC

37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21049

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/435, 14/705; C09K 11/06; G01N 33/53, 33/566

US CL :252/301.16, 301.36, 301.4R; 435/7.8; 530/350, 358

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 252/ 301.16, 301.32, 301.4R; 435/7.8; 530/350, 358

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	ZHOU et al. Nuclear receptors have distinct affinities for coactivators: characterization by fluorescence resonance energy transfer. Molecular Endocrinology. October 1998. Vol. 12, No. 10, pages 1594-1604, especially page 1596 and figures 1-4.	1-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*g* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

26 DECEMBER 1998

Date of mailing of the international search report

21 JAN 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

MICHAEL D. PAK

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21049

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, BIOSCIENCE, BIOSIS, CAPLUS, SCISEARCH

search terms: nuclear receptor, steroid receptor, retinoic acid receptor, co-activator, fret